The American Journal of Medicine

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How you can prevent attacks of angina pectoris

Three new studies have recently been added to the extensive investigation of Peritrate's effectiveness in preventing attacks of angina pectoris:

For some patients, state Rosenberg and Michelson, Peritrate "may mean the difference between complete, or almost complete, absence of symptoms, or a prolonged illness with much suffering." *Am. J. M. Sc.* 230:*254 (Sept.) 1955.*

"Impressive and sustained improvement" was also observed in a small number of patients treated by Kory *et al. Am. Heart J.* 50:*308 (Aug.) 1955.*

Among anginal prophylactic drugs evaluated by Russek's group "only this agent [Peritrate] appears worthy of the designation, 'long-acting coronary vasodilator.'" *Circulation* 12:*169 (Aug.) 1955.*

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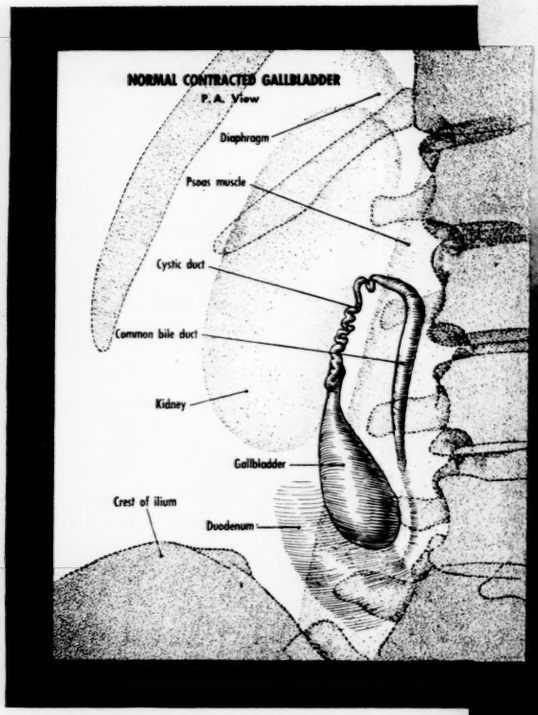

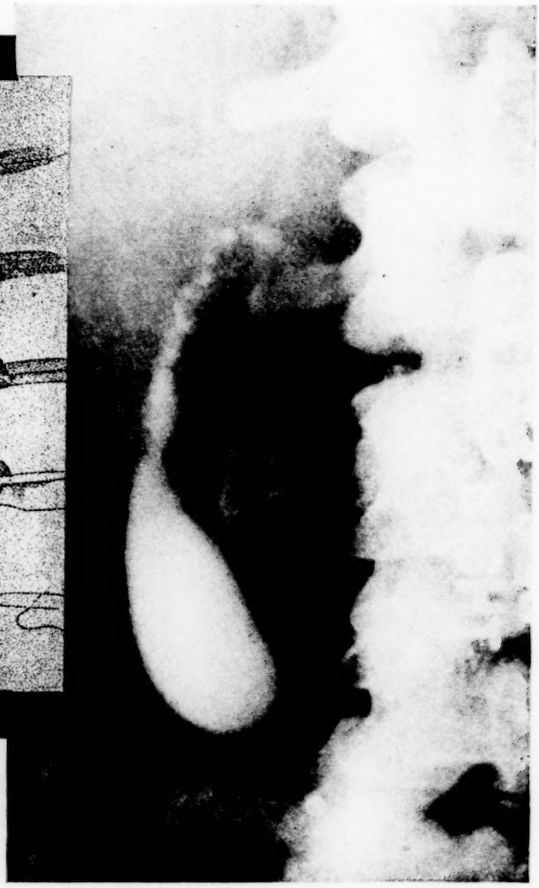

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Buckstein, Jacob: The Digestive Tract in Roentgenology. Philadelphia, J. B. Lippincott Co., 2nd ed., 1953, vol. 2, p. 1003.

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The American Journal of Medicine

Vol. XX JANUARY, 1956 No. 1

Editorial

Clinical Studies

The distinction between primary and secondary thyroid deficiency may be difficult to make on clinical grounds alone and often requires recourse to laboratory evidence for diagnosis or confirmation of diagnosis. The authors evaluate the several available laboratory tests on the basis of a very large experience in several hospitals, paying special attention to assay of the urinary gonadotropin (FSH) excretion as well as to thyroid response to thyrotropin administration. The collective data bring out a number of points of unusual intrinsic and practical diagnostic interest. Not the least intriguing are the problems arising from incomplete pituitary deficiencies and partial preservation of thyroid function in some patients with Simmonds' disease.

With the development of more effective methods of dealing with adrenal adenomas or hyperplasia, it has become a matter of some practical importance to distinguish these causes of Cushing's syndrome from adrenal carcinoma. The authors find that this may be possible by determinations of plasma 17-hydroxycorticosteroids and urinary 17-hydroxycorticoids and 17-ketosteroids, and the response of these levels to stimulation by administered ACTH. One case of adrenal carcinoma gave evidence of autonomous steroid production whereas one patient each with adrenal adenoma and hyperplasia showed a response to ACTH stimulation. The method would seem to deserve further exploration.

The authors describe three cases of Addison's disease associated with histoplasmosis and emphasize the importance of considering this etiology particularly in patients who have sojourned in highly endemic areas. Of interest is the success of the authors in controlling both diseases by appropriate measures.

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VOLUME TWENTY NUMBER ONE

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the drug of choice

. . . as a tranquilizing (ataractic) agent*
. . . in anxiety and tension states
. . . in hypertension

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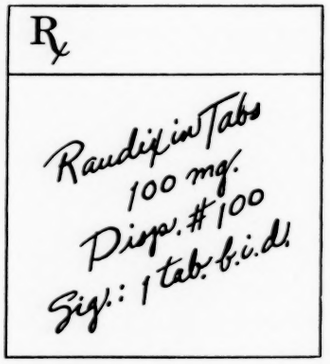

*Ataractic, from ataraxia: calmness untroubled by mental or emotional excitation. (Use of term suggested by Dr. Howard Fabing at a recent meeting of the American Psychiatric Association.)

SQUIBB

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VOLUME TWENTY NUMBER ONE

Review

The thoughtful physician must be concerned with the significance as well as with the relief of pain in his patients and in this connection the present discussion will be found of great interest. Dr. Beecher makes his points forcefully and lucidly. He emphasizes the importance of the reaction component, as opposed to the sensation stimulus, in the stimulation-suffering-relief sequence. He is dubious as to the propriety of equating, in man, pain produced by current experimental procedures with pain associated with pathologic processes of serious import. He points out that drugs given for pain, like placebos, affect the reaction and not the stimulus. Altogether a provocative and thought-stimulating essay well worth perusal.

Seminar on Allergy

The seminars on allergy are introduced by this review of present knowledge of the mechanisms and sites of synthesis of antibodies. The authors begin with a consideration of the relationship between the antibodies of the blood plasma and the plasma gamma globulins, current methods of study of this relationship and the chemical processes by which proteins are endowed with antibody specificity. They then discuss at length the vexing problem of the cellular sources of antibodies, in particular whether derived from plasma cells, lymphocytes or precursors of these cells, common or specific. It is concluded that there is valid evidence for participation of each of these cell types under various circumstances.

Clinico-pathologic Conference

Case Reports

Two cases of American mucocutaneous leishmaniasis (espundia) are described, each showing unequivocal evidence of visceral involvement. Systemic dissemination has not previously been emphasized in the literature but probably occurs more often than is appreciated. The authors properly question the sharp separation in classification of this form of leishmaniasis from the Sudan variant of the disease.

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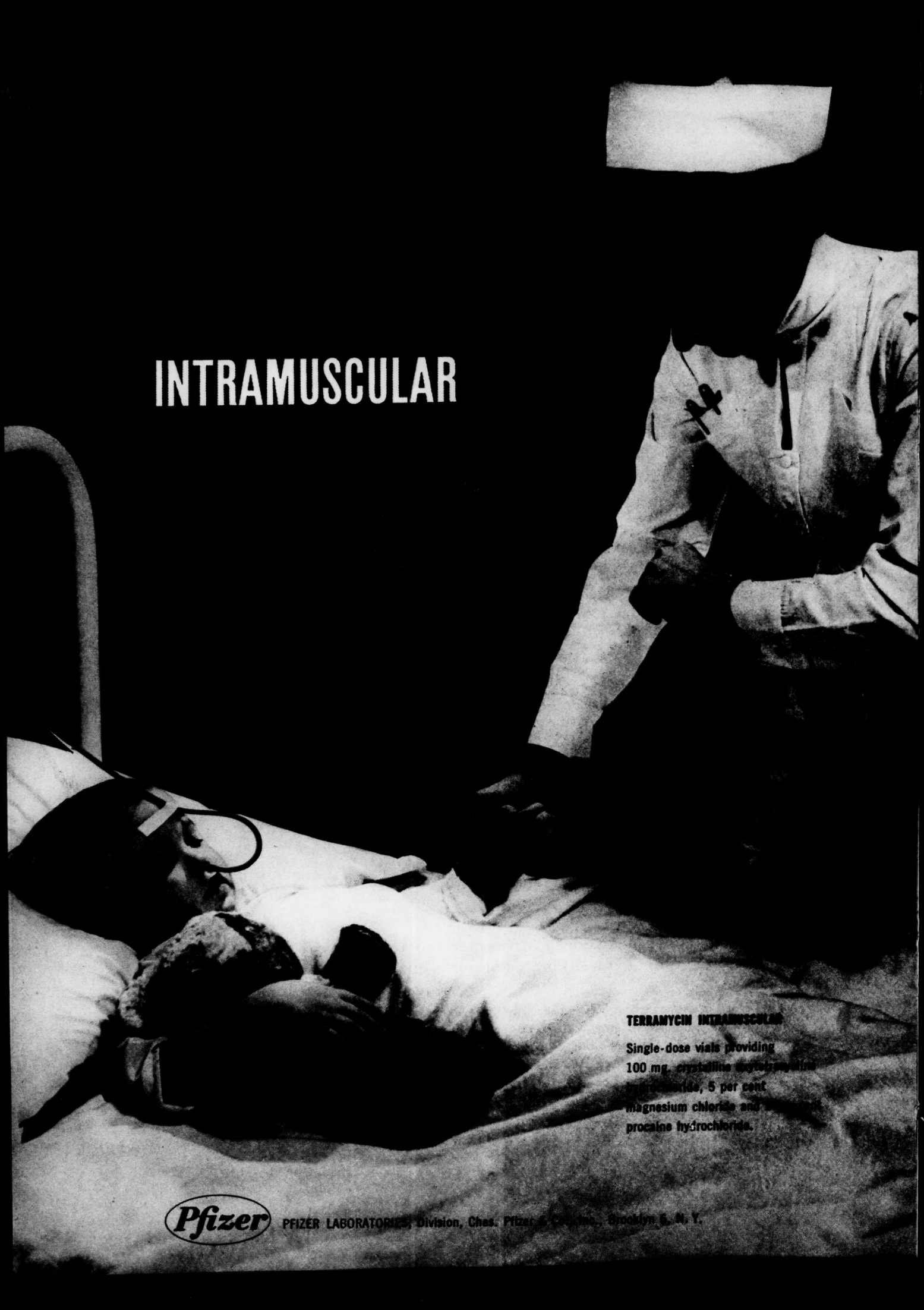
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Advertising Index on 3rd Cover

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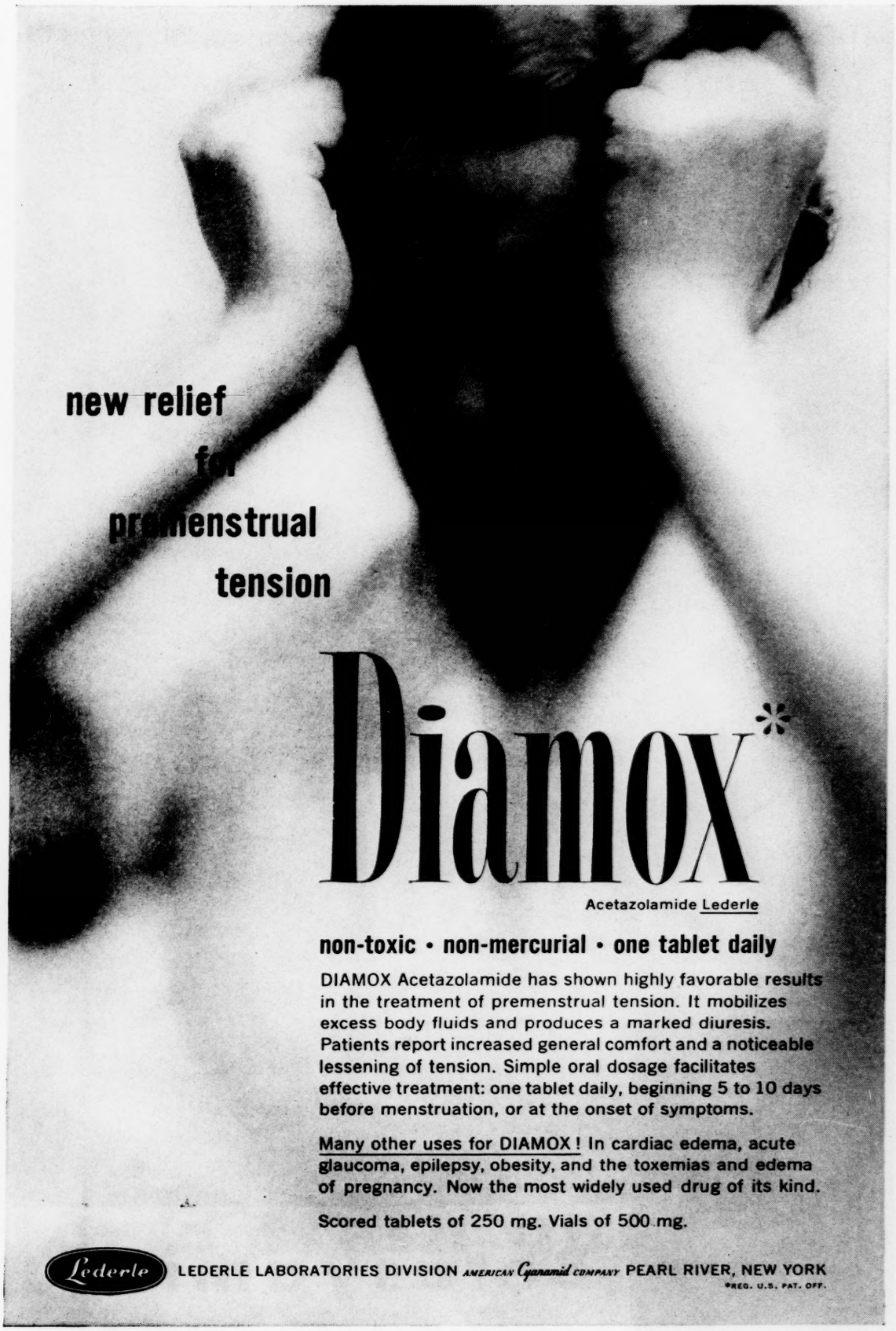
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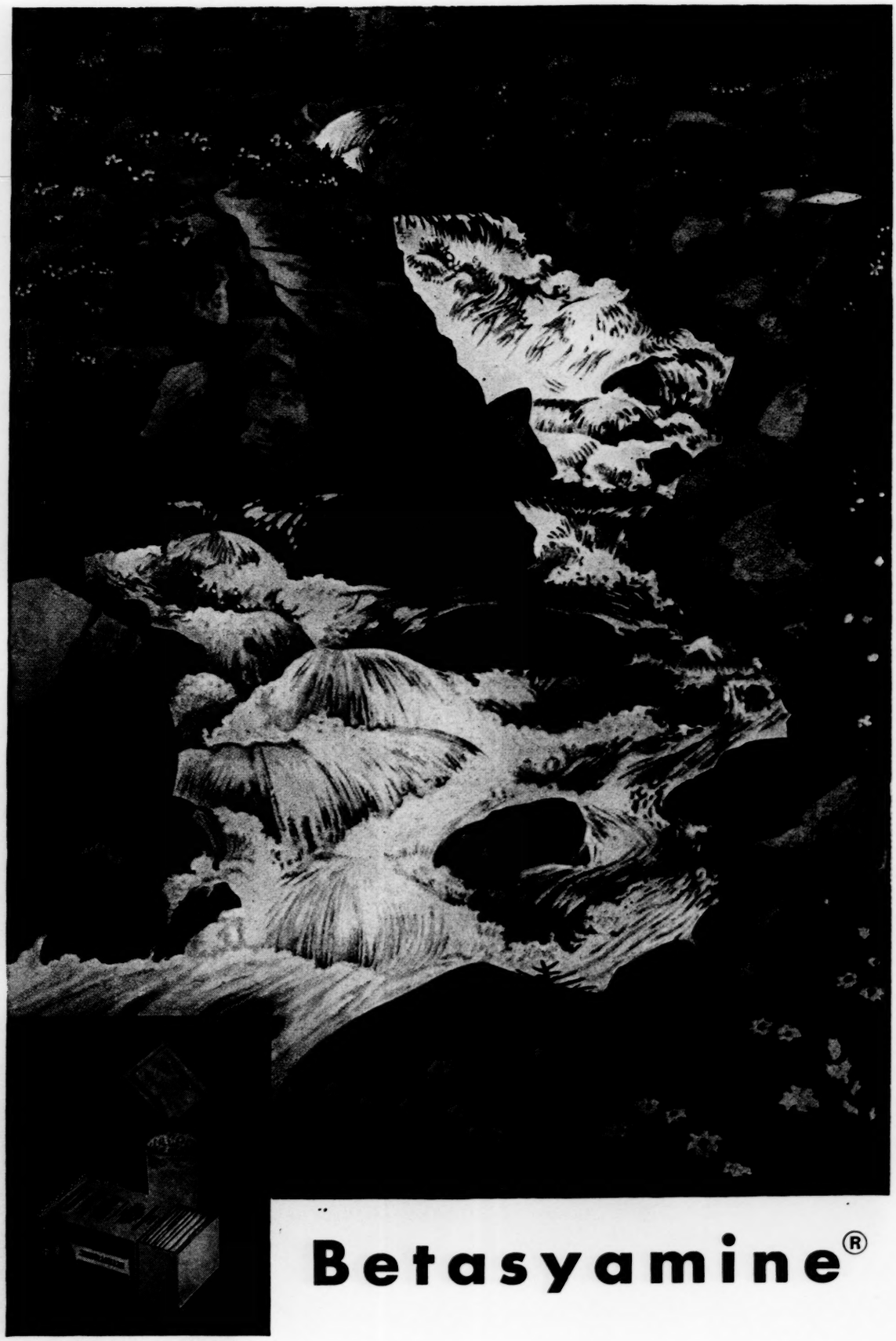

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1. Hirsch, S.: New York J. Med. *55:1170* (April 15) 1955. 2. Dixon, H. H.; and others: West. J. Surg. *60:327* (July) 1952).

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non-narcotic, non-opiate, highly palatable antitussive

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TOCLASE TABLETS. For convenience at work or recreation. 25 mg. tablets, bottles of 25.

*Trademark

1. Parish, F. A.: M. Times *83*:870 (Sept.) 1955.

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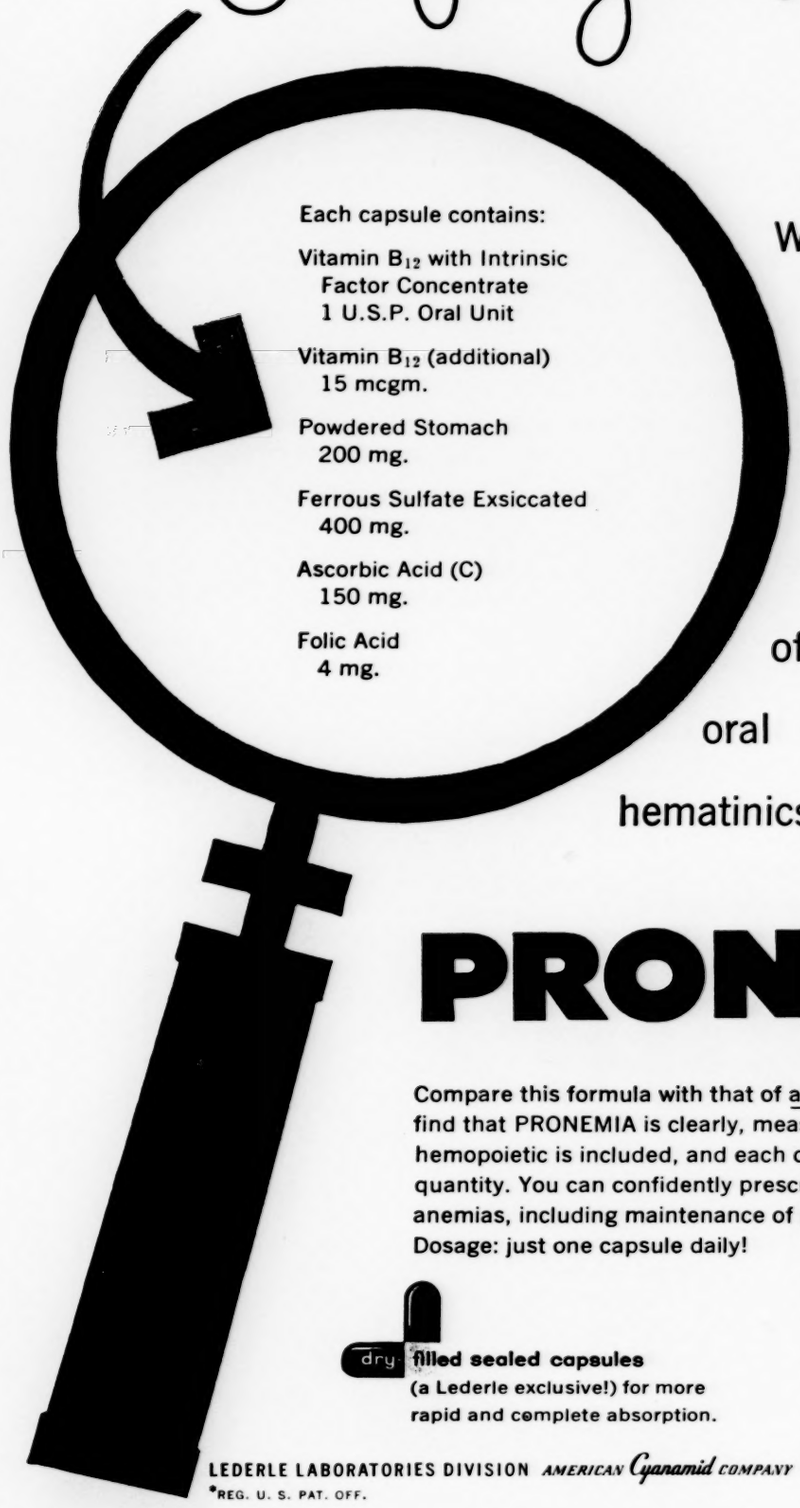

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Erythromycin in treatment of abscess

6/21/55

DISCHARGE SUMMARY

On 5/23/55 this patient (colored female, age 24) underwent an excisional biopsy of a breast tumor. On 5/24 tumor was removed and patient discharged from hospital on following day.

On 6/3/55 patient was readmitted because of purulent discharge from wound. On 6/3 a hemolytic Staph. aureus (coag. +) was isolated from abscess with the following disk sensitivities: penicillin, 1.5 units; erythromycin, 10 mcg; tetracycline, 10 mcg. Patient was placed on penicillin, 600,000 units b.i.d. for 10 days. On this schedule patient improved but progress was unsatisfactory and wound continued to discharge small amount of purulent material.

On 6/13 penicillin was discontinued and erythromycin started in dosage of 200 mgm. q.i.d. By 6/17 the discharge had stopped and wound was completely healed by 6/19. Erythromycin was continued until the patient was discharged from hospital on 6/21. Temp. was normal throughout hospital stay.

Final diagnosis: breast abscess due to Staph. aureus.

Result: rapid and complete recovery on erythromycin following failure of penicillin.

Communication to Abbott Laboratories.

specific against coccic infections

Now, you can prescribe an antibiotic (*Filmtab* ERYTHROCIN) that provides *specific therapy* against staph-, strep- or pneumococci. Since these organisms cause most bacterial respiratory infections (and since they are the very organisms most sensitive to ERYTHROCIN) doesn't it make good sense to prescribe ERYTHROCIN when the infection is coccic?

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Since ERYTHROCIN is inactive against gram-negative organisms, it is less likely to alter intestinal flora—with an accompanying low incidence of side effects. Also, your patients seldom get the allergic reactions sometimes seen with penicillin. Or loss of accessory vitamins during ERYTHROCIN therapy. *Filmtab* ERYTHROCIN (100 and 250 mg.), bottles of 25 and 100. Abbott

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". . . . . *it is our feeling that, at present, this [phenindione] is the oral anticoagulant of choice.*"

Breneman, G. M., and Priest, E. McC.: Am. Heart J. 50:129-35, 1955.

". . . . . *phenindione seems to be a more satisfactory anticoagulant at this time.*"

Wood, J. E., Jr.; Beckwith, J. R., and Camp, J. L.: J.A.M.A. 159:635, 1955.

HEDULIN permits dependable prothrombin control with little risk of dangerous fluctuation

(Not a Coumarin Drug)

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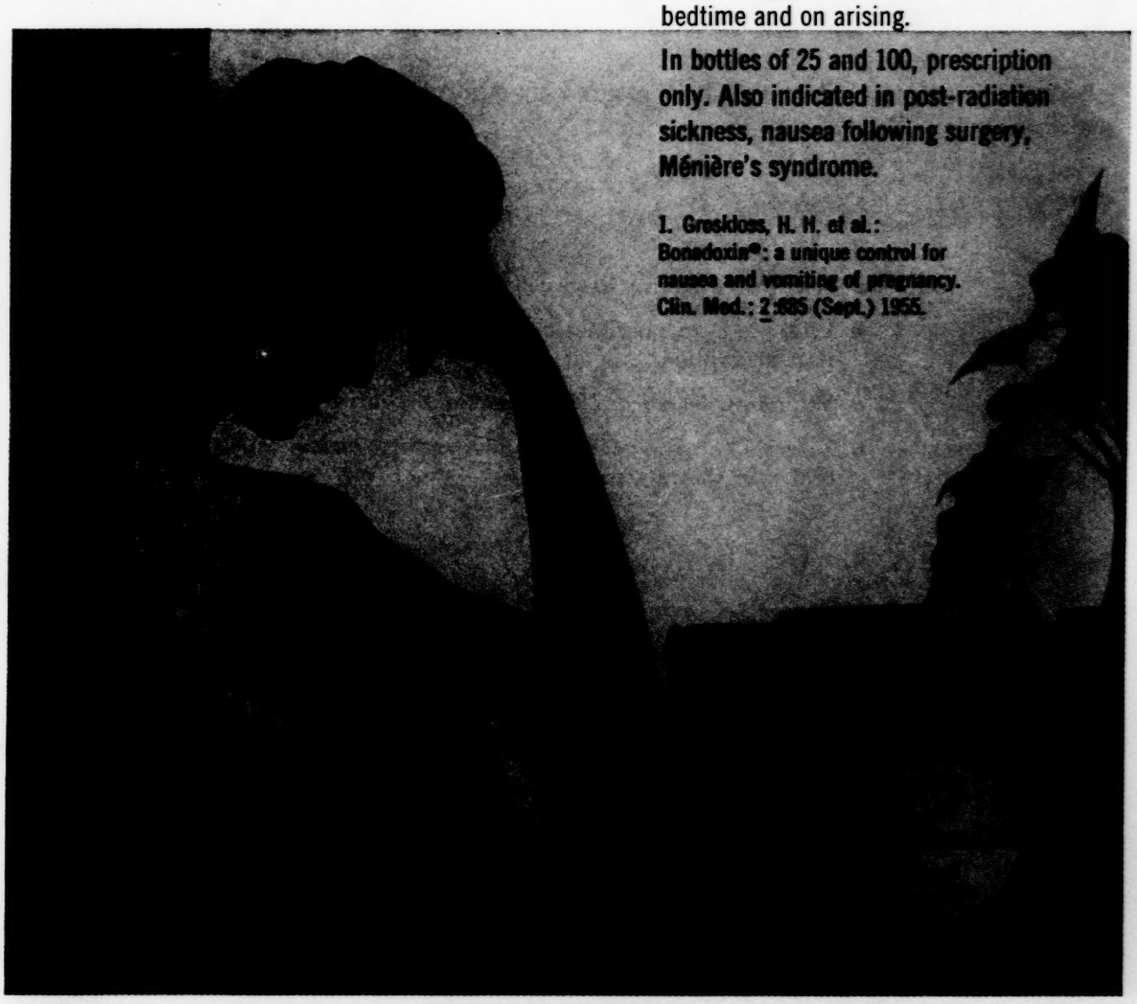

In bottles of 25 and 100, prescription only. Also indicated in post-radiation sickness, nausea following surgery, Ménière's syndrome.

1. Greskloss, H. H. et al.: Bonadoxin®: a unique control for nausea and vomiting of pregnancy. Clin. Med.: 2:835 (Sept.) 1955.

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*Sacks, M. S.: Ann. Int. Med. *42*: 458 (Feb.) 1955.

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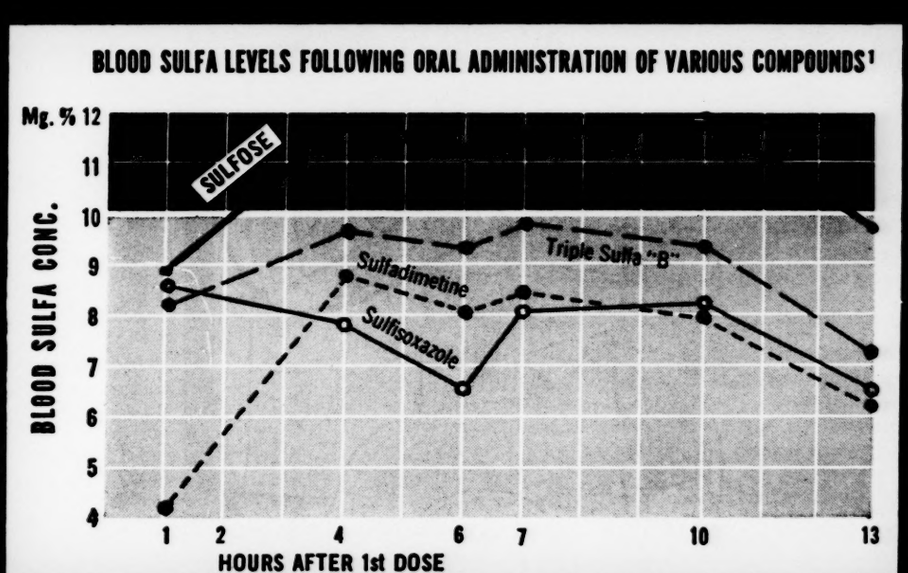

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[1] Beckowitz, D., Antibiot. & Chemo., 3:618, June 1953.

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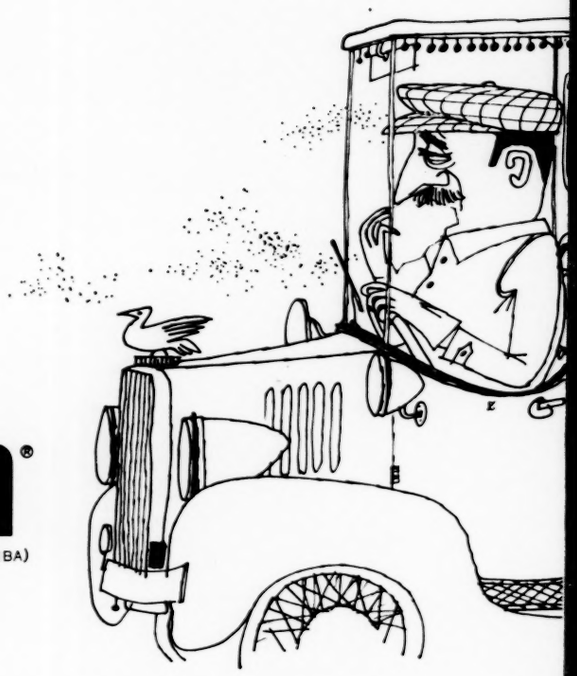

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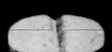
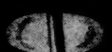
NEW  1 mg. tablet |  5 mg. tablet

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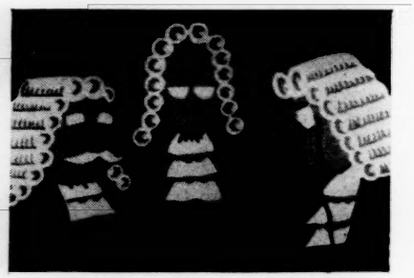

a "judicious combination..."

for antiarthritic therapy

SALCORT*

That cortisone and the salicylates have a complementary action has been well established.[1-5] In rheumatic conditions, functional improvement and a sense of feeling well are noted early. No withdrawal reactions have been reported.

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EACH TABLET CONTAINS:

Cortisone acetate	2.5 mg.
Sodium salicylate	0.3 Gm.
Aluminum hydroxide gel, dried .	0.12 Gm.
Calcium ascorbate	60 mg.
(equivalent to 50 mg. ascorbic acid)	
Calcium carbonate	60 mg.

* U.S. Pat. 2,691,662

1. Busse, E.A.: Treatment of Rheumatoid Arthritis by a Combination of Cortisone and Salicylates. Clinical Med. 11:1105 (Nov., 1955).
2. Roskam, J., VanCawenberge, H.: Abst. in J.A.M.A., 151:248 (1953).
3. Coventry, M.D.: Proc. Staff Meet., Mayo Clinic, 29:60 (1954).
4. Holt, K.S., et al.: Lancet, 2:1144 (1954).
5. Spies, T.D., et al.: J.A.M.A., 159:645 (Oct. 15, 1955).

BRISTOL, TENNESSEE
NEW YORK
KANSAS CITY
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The S. E. Massengill company

H^+ $\overline{OH}$

Superior antacid action and...

"For palatability, many patients prefer Maalox"[1]

MAALOX®, an efficient antacid suspension of magnesium-aluminum hydroxide gel, is smooth-textured, and always pleasant to take. MAALOX was tested by thousands of hospital outpatients, who preferred it to other antacids. Indeed, *high patient acceptability* (without danger of constipation) is one of the outstanding advantages of MAALOX therapy.[2]

As to chemistry: MAALOX has more acid-binding capacity than aluminum hydroxide gel, and maintains its antacid effect twice as long.[3]

Supplied: *Suspension*, bottles of 12 fluidounces. *Tablets*, bottles of 100. Samples sent promptly on request.

1. Kramer, P.: Med. Clin. North America, 39:1381, Sept. 1955.
2. Morrison, Samuel: Am. J. Gastroenterology 22:309 (1954).
3. Rossett, N. E., Rice, M. L., Jr., Gastroenterology 26:490 (1954).

For Pain
try Ascriptin Tablets
(Aspirin buffered with Maalox)
- Doubles blood salicylate level
- Action more prolonged
- High gastric tolerance level
- Clinically proved.

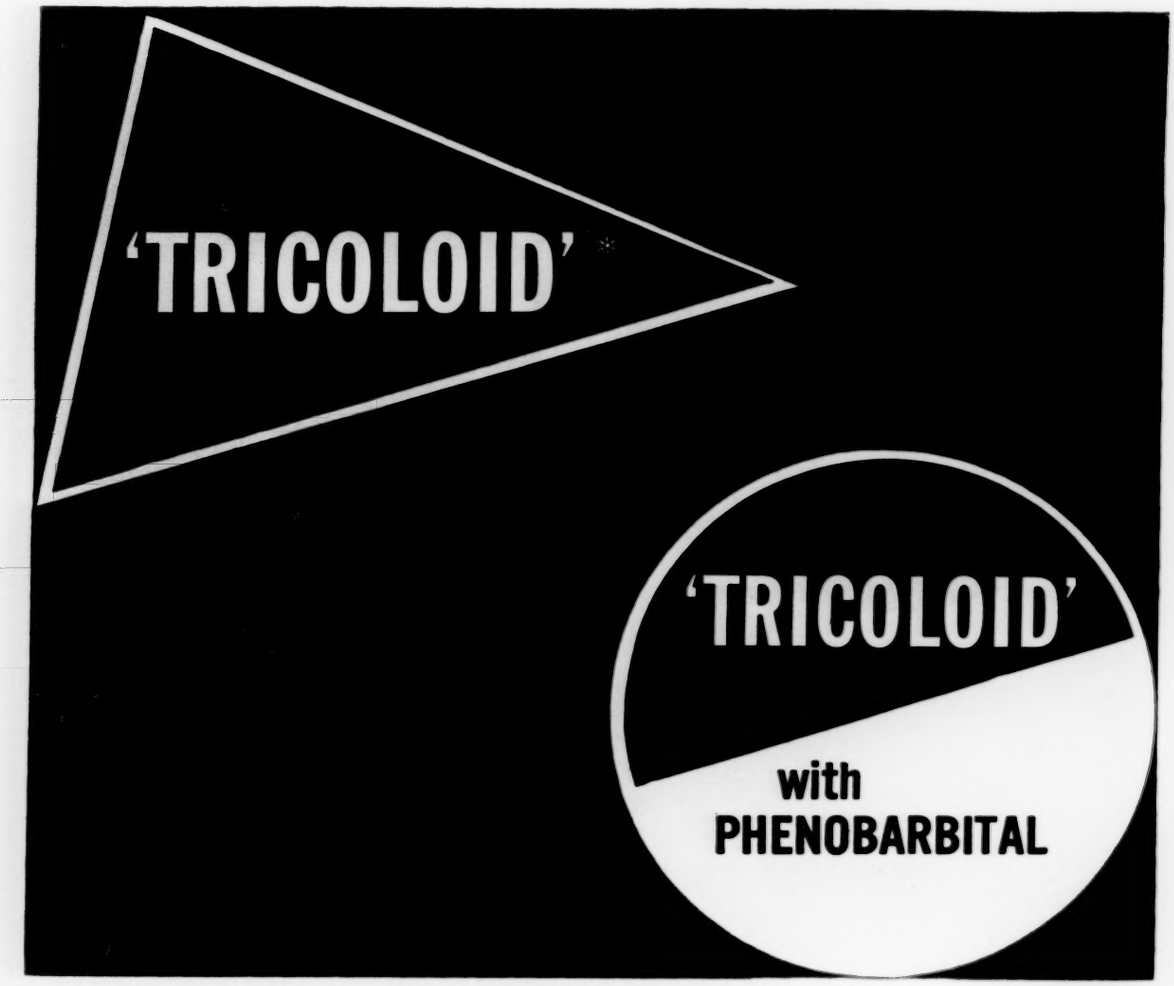
Samples on request.

Ascriptin

Maalox®

"... better suited for antacid therapy"[2]

WILLIAM H. RORER, Inc. RORER PHILADELPHIA, PA.



'TRICOLOID' or **'TRICOLOID' with Phenobarbital** is indicated, according to the degree of emotional tension which accompanies the symptoms, for the medical management of:

"lower bowel syndrome,"
nervous indigestion,
functional gastroenteritis,
peptic ulcer

* **'TRICOLOID'** *brand Tricyclamol 50 mg. Sugar-coated tablets*
'TRICOLOID' *brand Tricyclamol 50 mg. with Phenobarbital 16 mg. (gr. ¼) Sugar-coated tablets*

Both products in bottles of 100 and 1,000

BURROUGHS WELLCOME & CO. (U.S.A.) INC., *Tuckahoe, New York*

hasten convalescence with

STRESSCAPS*

Stress Formula Vitamins *Lederle*

STRESSCAPS provide those vitamins suggested by the National Research Council for use in conditions of stress. They provide nutritional supplementation in cases of shock, trauma, burns, fractures, etc.

STRESSCAPS promote wound healing, and stimulate antibody production as well as providing a nutritive reserve of water-soluble vitamins.

EACH CAPSULE CONTAINS:

Thiamine Mononitrate (B_1)	10 mg.
Riboflavin (B_2)	10 mg.
Niacinamide	100 mg.
Ascorbic Acid (C)	300 mg.
Pyridoxine HCl (B_6)	2 mg.
Vitamin B_{12}	4 mcgm.
Folic Acid	1.5 mg.
Calcium Pantothenate	20 mg.
Vitamin K (Menadione)	2 mg.

STRESSCAPS are supplied in a dry-filled sealed capsule, thereby eliminating any distasteful fats or oils and unpalatable after-taste.

AVERAGE DOSE: 1-2 capsules daily depending upon the severity of the condition.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY PEARL RIVER, NEW YORK Lederle

*REG. U.S. PAT. OFF.

new...paired piperidol action
for functional gastrointestinal complaints

rapid
visceral eutonic, Dactil®

prolonged
cholinolytic, Piptal®

relief throughout the G. I. tract

Tridal

TRIDAL permits more comprehensive control of gastrointestinal complaints by providing the combined benefits of two piperidols. The local action of Dactil* works immediately to give **rapid** relief of gastrointestinal pain and spasm; the potent cholinolytic Piptal† reinforces relief and provides **prolonged** normalization of secretion and motility.

TRIDAL is singularly free from urinary retention, constipation, dry mouth, blurred vision.

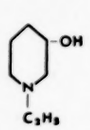
dosage: One TRIDAL Tablet two or three times a day and at bedtime. Unless rapidly swallowed with water, TRIDAL will produce some lingual anesthesia.

Each TRIDAL Tablet contains 50 mg. of Dactil and 5 mg. of Piptal. Bottles of 50 compressed, uncoated tablets.

*Dactil (the ***only*** brand of N-ethyl-3-piperidyl diphenylacetate hydrochloride): the piperidol to prescribe alone when no interference with digestive secretion is desired.

†Piptal (the ***only*** brand of N-ethyl-3-piperidyl-benzilate methobromide): the piperidol to prescribe alone when peptic ulcer is known to be present and normalization of secretion as well as motility is desired.

Lakeside Laboratories, Inc. PIONEERS IN PIPERIDOLS MILWAUKEE 1, WISCONSIN



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your best bet!

NOW...
Federal law permits oral prescription

Hycodan*

(Dihydrocodeinone with Homatropine Methylbromide)

BETTER THAN CODEINE **FOR COUGH**[1]

BETTER THAN CODEINE *PLUS* APC **FOR PAIN**[2]

Percodan

(Salts of Dihydrohydroxycodeinone and Homatropine plus APC)

Syrup and oral tablets. Each teaspoontul or tablet of HYCODAN contains 5 mg. dihydrocodeinone bitartrate and 1.5 mg. Mesopin. May be habit-forming. Average adult dose, 1 teaspoontul or 1 tablet after meals and at bedtime.

FASTER
•
LONGER-LASTING
•
MORE THOROUGH

Scored, yellow oral tablets. May be habit-forming. Average adult dose, 1 tablet q. 6 h.

Endo®

Literature Sent

ENDO PRODUCTS INC.
RICHMOND HILL 18, NEW YORK

New urethral suppositories relieve pain and fight infection

For the patient, "The treatment is shorter and more comfortable than with the conventional method..."*

- suppository melts and releases anesthetic agent to relieve pain and burning *rapidly*
- kills most bacteria common to urinary tract
- easy for patient to insert at home
- *safe*—irritation rare in over 340 reported cases*
- *proved*—"The suppository method of medication has proved its worth."*. . . in bacterial (granular) urethritis; for prophylaxis and pain-relief before and after instrumentation.

to prevent cross-infection...

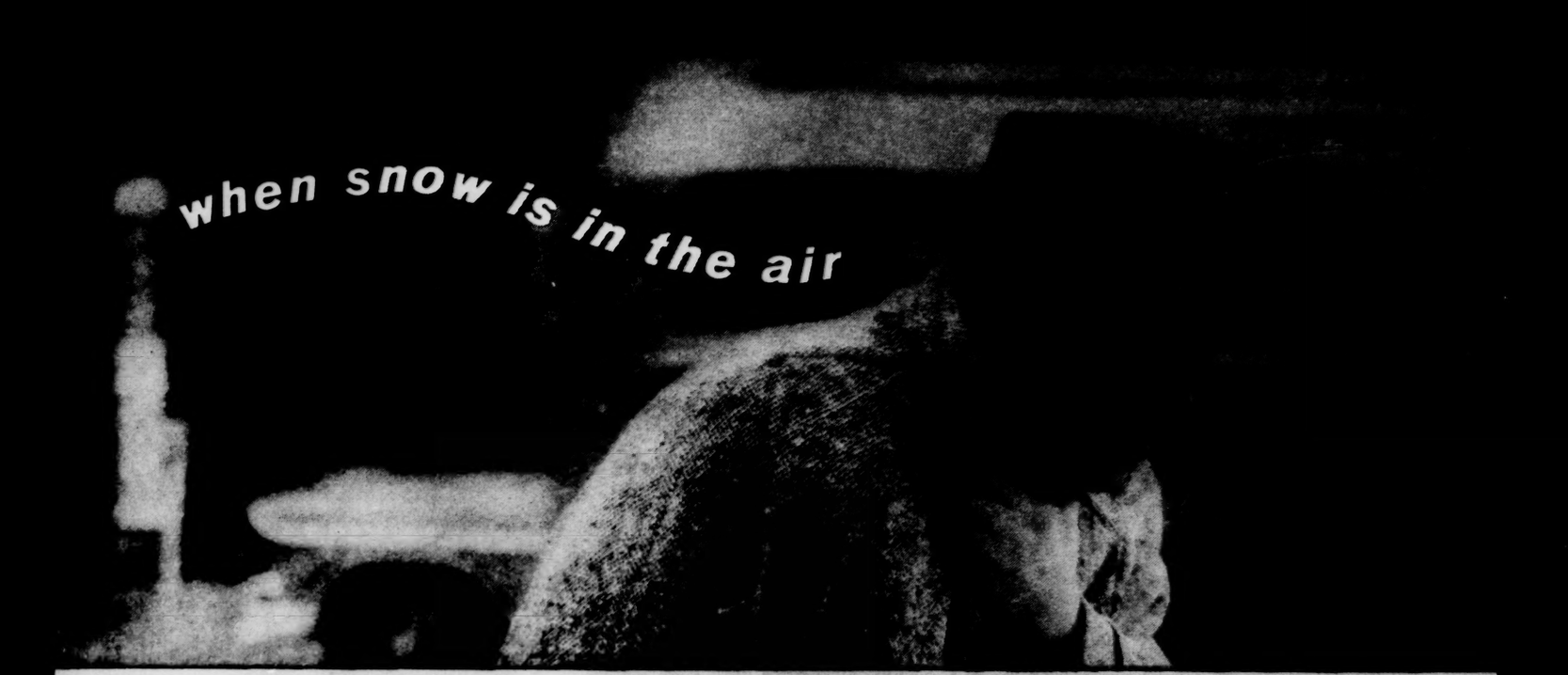
FURACIN® vaginal Suppositories are used with Furacin Urethral Suppositories to prevent cross-infection from the vagina. Hermetically sealed in yellow foil. Box of 12.

*Youngblood, V. H.: J. Urol. *70*:926, 1953.

FURACIN®
BRAND OF NITROFURAZONE
urethral
SUPPOSITORIES

Each Furacin Urethral Suppository contains 0.2% Furacin (brand of nitrofurazone) and 2% diperodon HCl in a water-miscible base. Hermetically sealed in silver foil. Box of 12.

EATON LABORATORIES, Norwich, N.Y. **NITROFURANS** a new class of antimicrobials neither antibiotics nor sulfas



time for

Tyzine®

brand of tetrahydrozoline hydrochloride

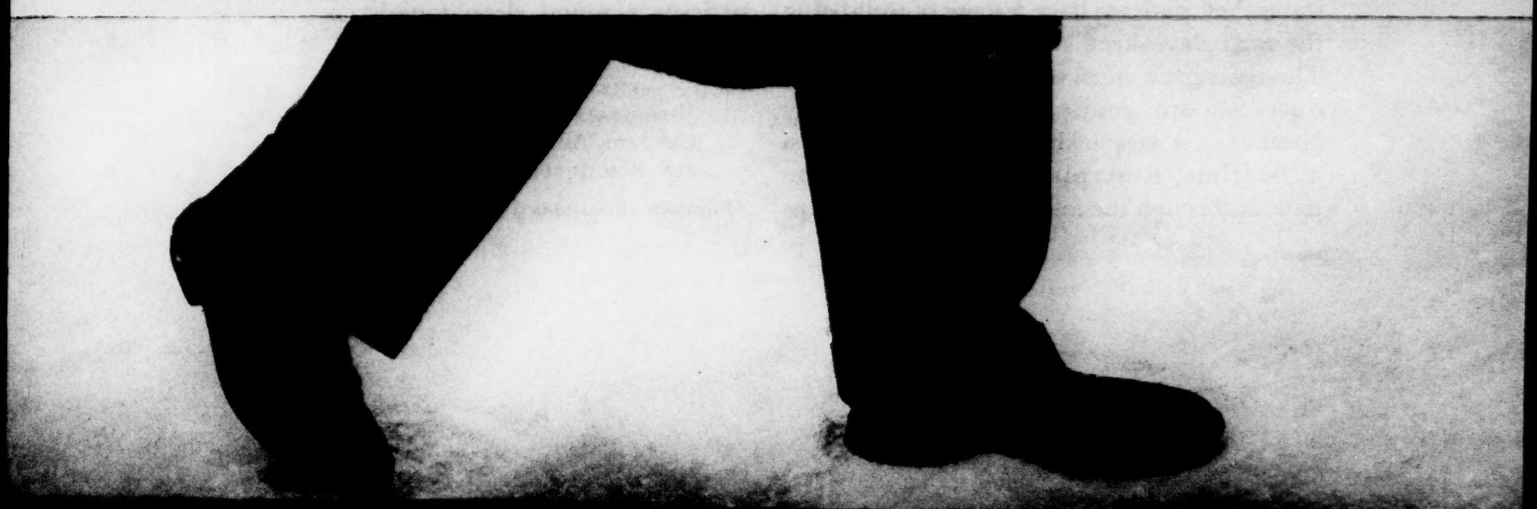
nasal decongestion in minutes for hours

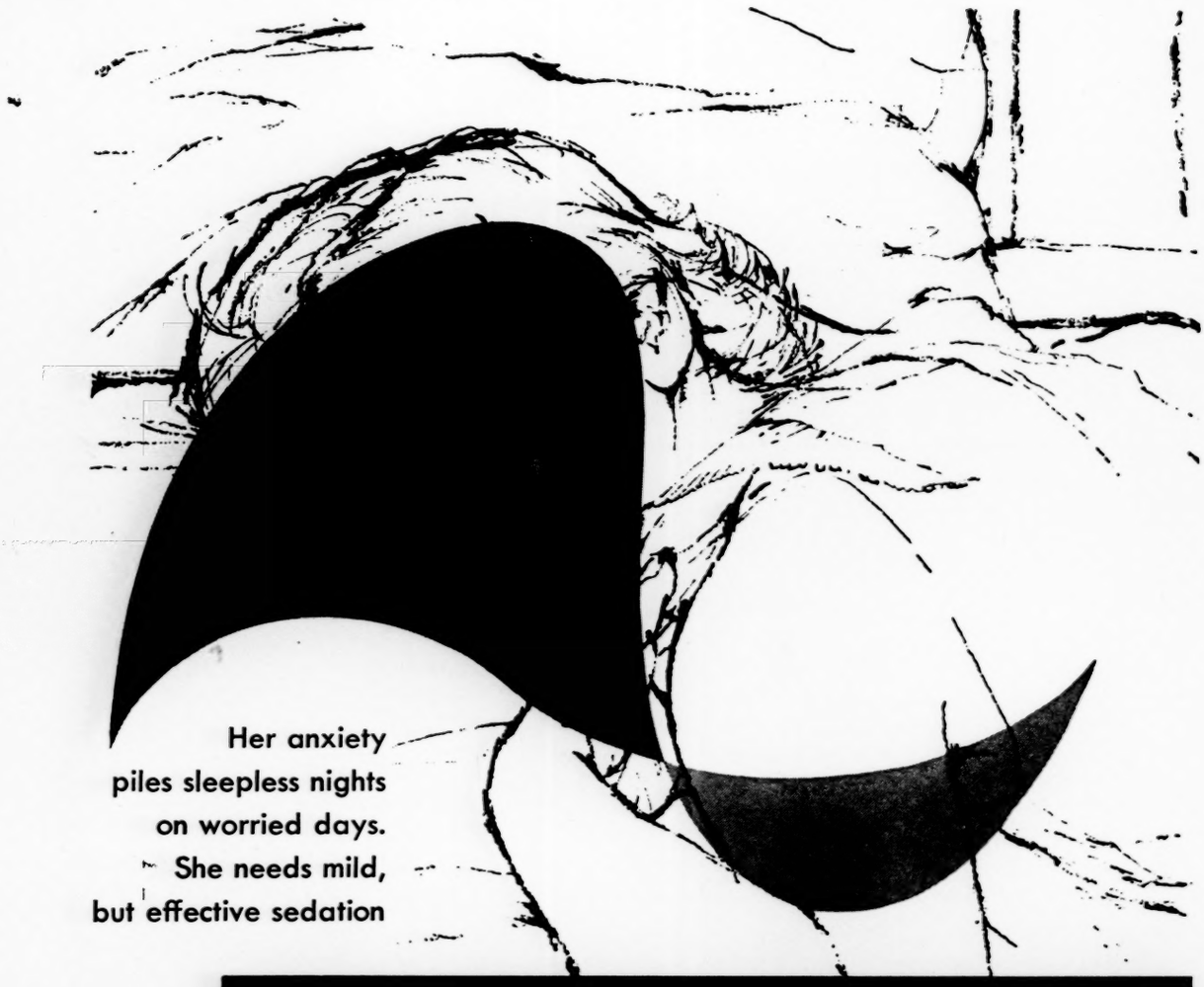
"a new and superior topical vasoconstrictor,"* with effectiveness lasting even up to 6 hours, without rebound engorgement. Odorless, tasteless, free of sting, burn, irritation, and CNS stimulation.

Supplied: TYZINE Nasal Spray, in plastic bottles, 15 cc. TYZINE, 0.1%. TYZINE Nasal Solution, in 1-oz. dropper bottles, 0.1%. TYZINE Pediatric Nasal Drops, in 1/2-oz. bottles, 0.05%, with calibrated dropper for precise dosage.

**Menger, H. C.: New York J. Med. 55:812, 1955*

PFIZER LABORATORIES *Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York* Pfizer





for a sound sleep tonight, a calm day tomorrow . . .

filmtab

Nembu-Serpin

TRADEMARK

Just one tablet at bedtime of this new combination of NEMBUTAL and Reserpine will calm the worries of most patients with mild anxiety states. Yet, patients have a sense of well-being the next day—keep their drive and energy. The synergistic effect of the combination produces smooth, gentle, prolonged sedation: NEMBUTAL acts quickly to induce drowsiness at bedtime, Reserpine sedation calms the patient through the following day. Small dosage makes side effects rare. Also recommended for treatment of mild essential hypertension. Prescribe NEMBU-SERPIN to give your anxiety patients a sound sleep tonight, a calm day tomorrow. In bottles of 100. Abbott

Each NEMBU-SERPIN Filmtab contains—
NEMBUTAL® Calcium (Pentobarbital Calcium, Abbott), 30 mg. (½ gr.)
and Reserpine, 0.25 mg.

®Filmtab—Film-sealed tablets; pat. applied for.

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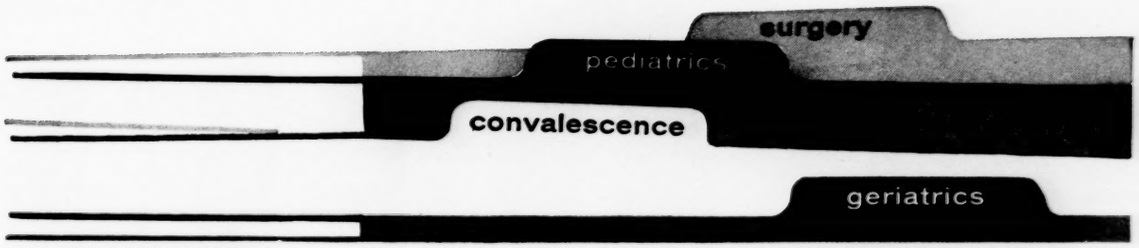

LIVITAMIN® with IRON
each fluidounce contains:

Iron peptonized ..... 420 mg.
(Equiv. in elemental iron to 70 mg.)
Manganese citrate, soluble ..... 158 mg.
Thiamine hydrochloride ..... 10 mg.
Riboflavin ..... 10 mg.
Vitamin B_{12} (crystalline) ..... 20 mcg.
Niacinamide ..... 50 mg.
Pyridoxine hydrochloride ..... 1 mg.
Pantothenic acid ..... 5 mg.
Liver fraction 1 ..... 2 Gm.
Rice bran extract ..... 1 Gm.
Inositol ..... 30 mg.
Choline ..... 60 mg.

. . . the reconstructive iron tonic of wide application . . .

LIVITAMIN®

WITH IRON

In debilitation, syndrome therapy instead of symptom treatment is required. Livitamin (Massengill) provides comprehensive therapy and adequate nutritional support. The appetite improves, as does the blood picture . . . improved anabolism and better digestion produce a significant syndrome reversal.

LIVITAMIN® CAPSULES with INTRINSIC FACTOR
each capsule contains:

Desiccated liver ..... 450 mg.
Ferrous sulfate ..... 130 mg.
(Equiv. to 25 mg. of elemental iron)
Thiamine hydrochloride ..... 3 mg.
Riboflavin ..... 3 mg.
Niacinamide ..... 10 mg.
Vitamin B_{12} ..... 5 mcg.
Pyridoxine hydrochloride ..... 0.5 mg.
Calcium pantothenate ..... 2 mg.
Folic acid ..... 1 mg.
Intrinsic factor USP ..... 1/6 Unit

. . . in pernicious anemia and geriatrics . . .

LIVITAMIN®

CAPSULES WITH INTRINSIC FACTOR

Intrinsic factor is essential to provide full utilization of antianemic and nutritional factors in P. A. and many Geriatric patients. Livitamin Capsules with Intrinsic Factor (Massengill) contain intrinsic factor, U.S.P., iron and the B-complex vitamins. This integrated medication provides an optimal response in these difficult patients.

THE S. E. MASSENGILL COMPANY
BRISTOL, TENNESSEE

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ACHROMYCIN*

Tetracycline *Lederle*

widely prescribed because of these important advantages:

1) rapid diffusion and penetration
2) prompt control of infection
3) true broad-spectrum activity (proved effective against a wide variety of infections caused by Gram-positive and Gram-negative bacteria, rickettsiae, and certain viruses and protozoa)
4) negligible side effects
5) every gram produced in Lederle's *own* laboratories under rigid quality control, and offered *only* under the Lederle label
6) a *complete* line of dosage forms

in prolonged illness, prescribe

ACHROMYCIN SF

TETRACYCLINE with STRESS FORMULA VITAMINS

Attacks the infection, bolsters the body's natural defense. Stress vitamin formula suggested by the National Research Council in *dry-filled, sealed* capsules with ACHROMYCIN, 250 mg. Also available: ACHROMYCIN SF ORAL SUSPENSION (Cherry Flavor), 125 mg. per 5 cc.

dry-filled sealed capsules

(a Lederle exclusive!) for more rapid and complete absorption. No oils, no paste, tamperproof!

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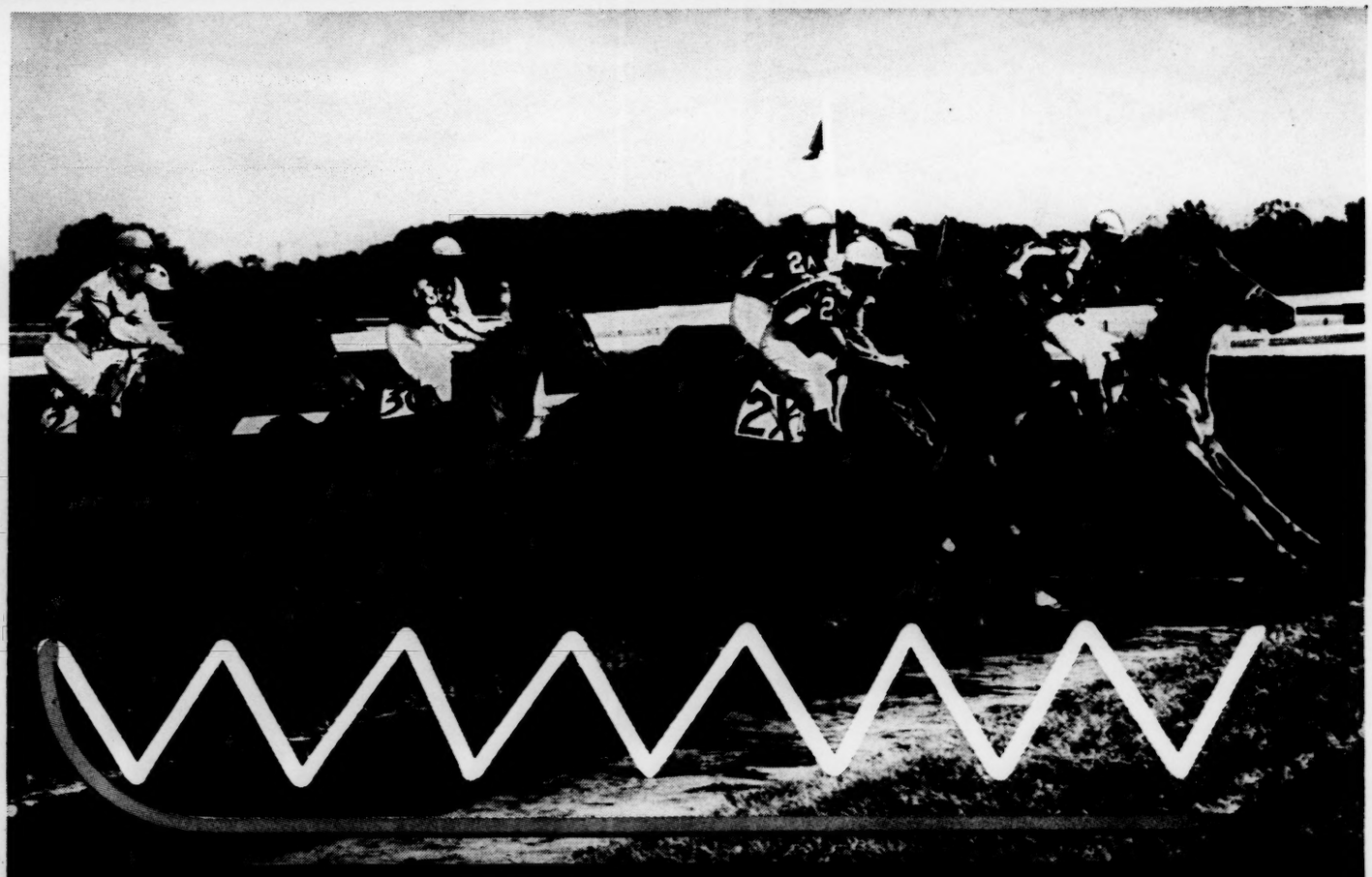


Edema Control Need Not Be a Steeplechase

Steeplechase diuresis is up-and-down diuresis: patient dry, patient waterlogged.

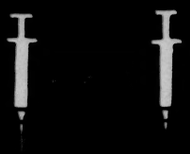
In contrast, the THIOMERIN regimen offers *straightline* control . . . with maximal *spread* between injections. Parenteral THIOMERIN to initiate diuresis is supplemented by THIOMERIN Suppositories to space out the injections, to prevent fluid accumulation, to maintain dry weight.

THIOMERIN Suppositories promote flat-plane management . . . with little likelihood of mercurialism, rectal irritation, or local discomfort.[1]

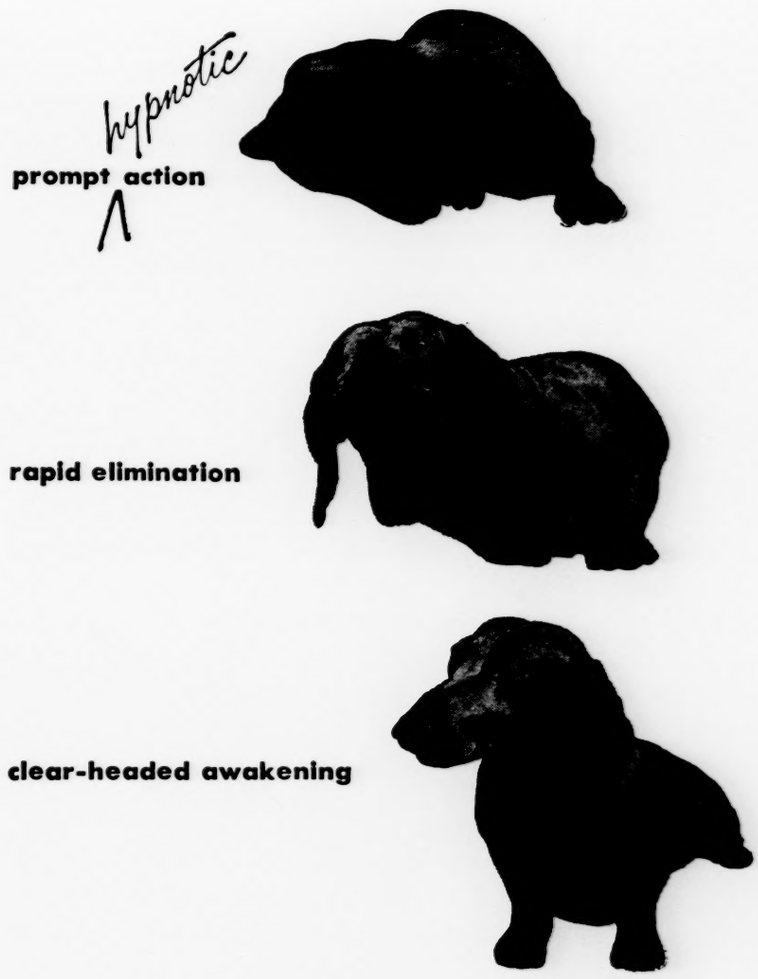
Supplied: THIOMERIN Suppositories, boxes of 12.
Also available: Injection THIOMERIN Solution, vials of 2 cc., boxes of 12; vials of 10 cc. Injection THIOMERIN Lyophilized, vials of 1.4 Gm.; vials of 4.2 Gm.

1. Daly, J.W.: Am. J. M. Sc. [illegible]:440, Oct., 1954

INJECTION [illegible]
THIOMERIN SODIUM
[illegible]



Wyeth



ELIXIR ALURATE 'Roche'

Available as ELIXIR ALURATE, cherry red color/ELIXIR ALURATE VERDUM, emerald green color
Each contains 0.03 Gm (½ grain) of Alurate per teaspoonful (4 cc)
in a palatable vehicle. Alurate®—brand of aprobarbital

HOFFMANN-LA ROCHE INC. • ROCHE PARK • NUTLEY 10 • NEW JERSEY

now...

a new fortified CORICIDIN

for an extra measure
of relief and comfort
even in severe colds

Each red and yellow capsule provides:

Chlorprophenpyridamine maleate	4 mg.
Salicylamide	190 mg.
Phenacetin	130 mg.
Caffeine	30 mg.
Ascorbic acid	50 mg.
Methamphetamine hydrochloride	1.25 mg.

On ℞ and cannot be refilled without your permission.
Bottles of 100 and 1000.

CORICIDIN,® brand of analgesic-antipyretic.

A penny saved is a penny ------

Something missing? Sure—that important last word!

When you prescribe prenatal capsules, the word to remember is *Lederle*. Write it, and assure your patient the genuine Lederle formula!

PRENATAL CAPSULES LEDERLE

Dosage: 1 to 3 capsules daily, throughout pregnancy and lactation.

Each capsule contains:

Vitamin A	2000 U.S.P. Units	Folic Acid	1 mg.
Vitamin D	400 U.S.P. Units	Calcium (in $CaHPO_4$)	250 mg.
Thiamine Mononitrate (B_1)	2 mg.	Phosphorus (in $CaHPO_4$)	190 mg.
Riboflavin (B_2)	2 mg.	Dicalcium Phosphate Anhydrous ($CaHPO_4$)	869 mg.
Niacinamide	7 mg.	Iron (in $FeSO_4$)	6 mg.
Vitamin B_{12}	1 mcgm.	Ferrous Sulfate Exsiccated	20 mg.
Vitamin K (Menadione)	0.5 mg.	Manganese (in $MnSO_4$)	0.12 mg.
Ascorbic Acid (C)	35 mg.		

dry-filled sealed capsules—a Lederle exclusive! More rapidly and completely absorbed. No oils, no paste . . . no aftertaste.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY PEARL RIVER, NEW YORK Lederle

THIS IS

Rauwiloid®

a preparation of choice in the treatment of HYPERTENSION

- Rauwiloid represents the balanced, mutually potentiated actions[1] of several Rauwolfia alkaloids, of which reserpine and the equally antihypertensive rescinnamine have been isolated.
- Hence, reserpine is not the total active antihypertensive principle of the rauwolfia plant.
- Rauwiloid, the alseroxylon fraction of Rauwolfia serpentina, Benth., is freed ot the undesirable alkaloids of the whole root. Recent investigations confirm the desirability of Rauwiloid (because of the balanced action of its contained alkaloids) over single alkaloidal preparations; "...mental depression...was...less frequent with alseroxylon..."[2]

The dose-response curve of Rauwiloid is flat, and its dosage is uncomplicated and easy to prescribe... merely two 2mg. tablets at bedtime.

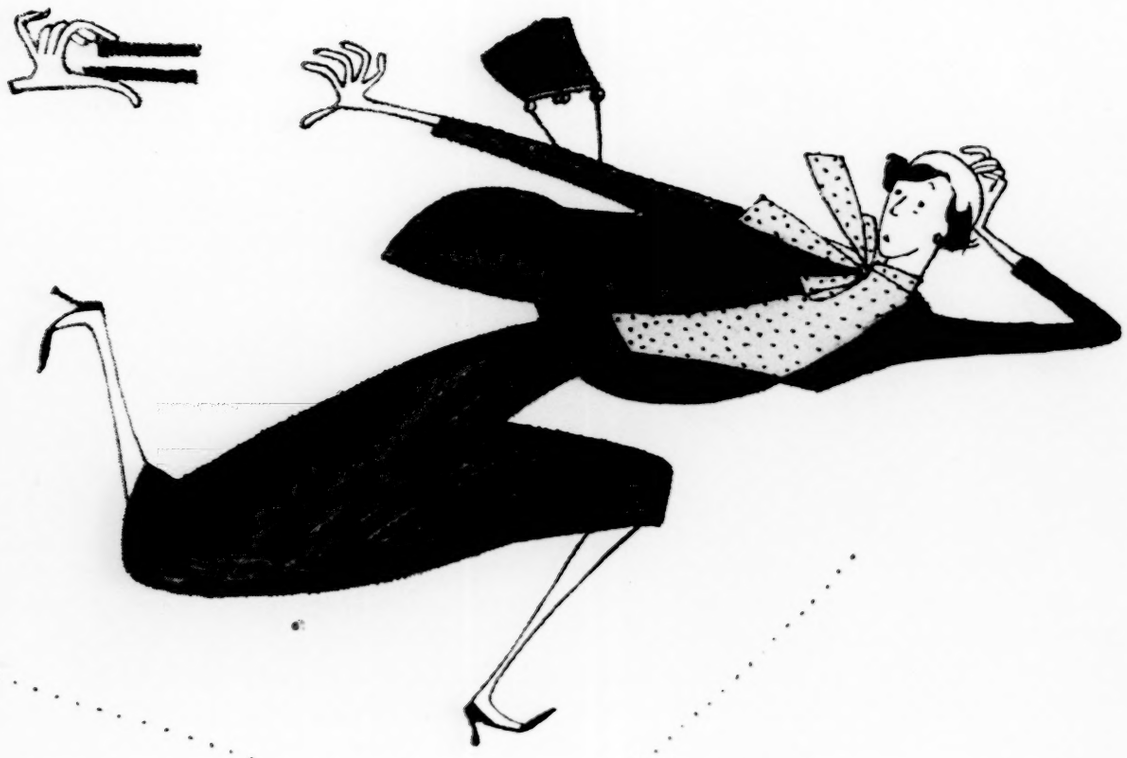
1. Cronheim, G., and Toekes, I.M.: Comparison of Sedative Properties of Single Alkaloids of Rauwolfia and Their Mixtures, Meeting of the American Society for Pharmacology and Experimental Therapeutics, Iowa City, Iowa, Sept. 5, 1955.

2. Moyer, J.H.; Dennis, E., and Ford, R.: Drug Therapy (Rauwolfia) of Hypertension. II. A Comparative Study of Different Extracts of Rauwolfia When Each Is Used Alone (Orally) for Therapy of Ambulatory Patients with Hypertension, A.M.A. Arch. Int. Med. *96*:530 (Oct.) 1955.

Riker

LOS ANGELES

Rauwiloid is the original alseroxylon fraction of India-grown Rauwolfia serpentina, Benth., a Riker research development.



quick—

what's a good

B complex?

sur-Bex® with C

(ABBOTT'S B COMPLEX TABLETS WITH C)

Each SUR-BEX with C tablet supplies:

Thiamine Mononitrate		6 mg.
Riboflavin		6 mg.
Nicotinamide		30 mg.
Pyridoxine Hydrochloride		1 mg.
Vitamin B_{12} (as vitamin B_{12} concentrate)		2 mcg.
Pantothenic Acid (as calcium pantothenate)		10 mg.
Ascorbic Acid		150 mg.
Liver Fraction 2, N.F	(5 grs.)	300 mg.
Brewer's Yeast, Dried	(2½ grs.)	150 mg.

Abbott

As a dietary supplement: 1 or 2 tablets daily.

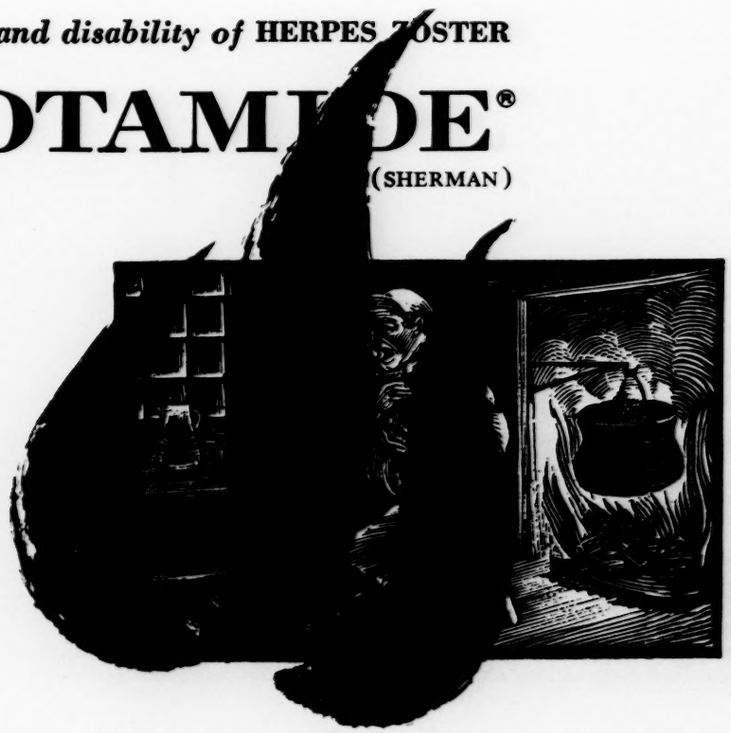
For stress, or postoperative convalescence: 2 or more tablets daily.

601186

for the pain and disability of **HERPES ZOSTER**

PROTAMIDE®

(SHERMAN)



published studies show:*

Improvement is "almost immediate," with "good to excellent results" in four out of five patients, and no postherpetic neuralgia in any patient who responded favorably.

Protamide is a sterile colloidal solution prepared from animal gastric mucosa . . . denatured to eliminate protein reaction . . . completely safe and virtually painless by intramuscular injection.

Clinical data on request.

use* PROTAMIDE® *first

in herpes zoster and post-infection neuritis

SHERMAN LABORATORIES

BIOLOGICALS • PHARMACEUTICALS

*Combes, F. C. & Canizares, O.: New York St. J. Med. 52:706, 1952; Marsh, W. C.: U. S. Armed Forces M. J. 1:1045, 1950.

broad spectrum antibiotic therapy, effective in many common infections

Because it contains Steclin (Squibb Tetracycline), MYSTECLIN is an effective therapeutic agent for most bacterial infections. When caused by tetracycline-susceptible organisms, the following infections are a few of those which can be expected to respond to MYSTECLIN therapy:

bronchitis • colitis • furunculosis • gonorrhea • lymphadenitis • meningitis • osteomyelitis • otitis media • pneumonia • pyelonephritis • sinusitis • tonsillitis

MYSTECLIN is also indicated in certain viral infections and in amebic dysentery.

broad spectrum antibiotic therapy, with a minimum of side effects

In clinical use, Steclin has produced an extremely low incidence of the gastrointestinal distress sometimes observed with other broad spectrum antibiotics. Mycostatin (Squibb Nystatin), as contained in MYSTECLIN, is also a particularly well tolerated antibiotic and has produced no allergic reactions, even after prolonged administration.

broad spectrum antibiotic therapy, without the danger of monilial overgrowth

Because it contains Mycostatin, the first safe antifungal antibiotic, MYSTECLIN effectively prevents the overgrowth of Candida albicans (monilia) frequently associated with the administration of ordinary broad spectrum antibiotics. This overgrowth may sometimes cause gastrointestinal distress, anal pruritus, vaginitis, and thrush; on occasion, it may have serious and even fatal consequences.

SQUIBB

1950	1952	1954	1955
Cortone®	Hydrocortone®	'Alflorone'	Deltra®

'Hydeltra'

5 mg.-2.5 mg.-1 mg.

(scored)

'Hydeltra' Indications:

'Hydeltra'

'Hydeltra'

SHARP & DOHME

NOW—EFFECTIVE STEROID HORMONE THERAPY OF RHEUMATIC AFFECTIONS WITH GREATER SAFETY AND ECONOMY

PABALATE-HC

Robins

Pabalate with Hydrocortisone

Clinical evidence indicates that, in Pabalate-HC, the synergistic antirheumatoid effects of hydrocortisone, salicylate, para-aminobenzoate, and ascorbic acid achieve satisfactory remission of symptoms in *up to 85% of cases studied*

—with a much higher degree of safety

—even when therapy is maintained for long periods

—at significant economy for the patient

Each tablet of Pabalate-HC contains 2.5 mg. of hydrocortisone — 50% more potent than cortisone, yet not more toxic.

FORMULA

In each tablet:

Hydrocortisone alcohol 2.5 mg.
Para-aminobenzoate 0.3 Gm.
Ascorbic acid 50.0 mg.

DOSAGE: Two tablets four times daily.
Additional information on request.

AVAILABLE FOR YOUR PRESCRIPTION NOW

A. H. ROBINS CO., INC. RICHMOND 20, VIRGINIA
Ethical Pharmaceuticals of Merit since 1878

THE COUGH STRUCK ONE...

Hickory, dickory, dock,
The cough went round the clock.

PHENERGAN Expectorant quiets cough and calms the patient through *four* distinct actions—topical anesthetic, antihistaminic, sedative, and expectorant. It is the round-the-clock preference of many clinicians, their agent of choice in cough control.

PHENERGAN®
EXPECTORANT

Promethazine Expectorant with Codeine
Plain (without Codeine)

Wyeth®
Philadelphia 1, Pa.

in pregnancy...a good nutritional start

NATABEC® KAPSEALS®

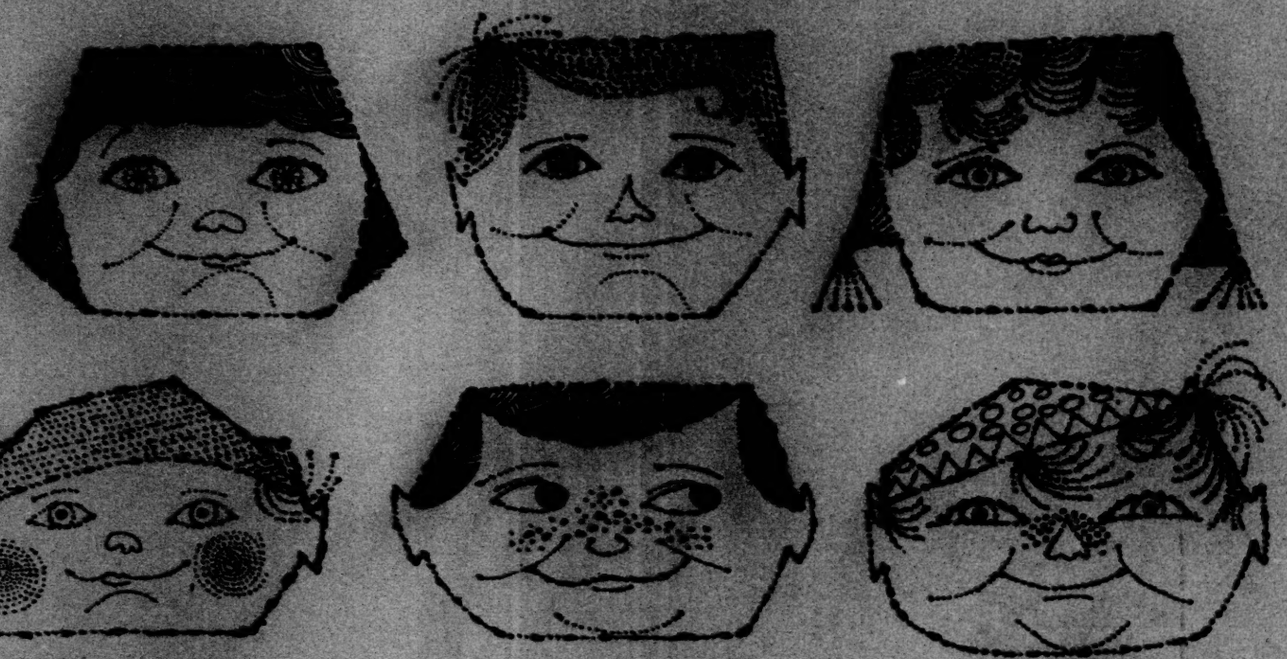
vitamin-mineral combination

Prescribed early in pregnancy, NATABEC Kapseals get your patients off to a good nutritional start—help keep vitamin-mineral intake abreast of increased nutritional needs. NATABEC Kapseals provide iron and calcium, as well as important vitamins in a formulation expressly designed to protect the health of both mother and child.

DOSAGE: As a dietary supplement during pregnancy and lactation, one or more Kapseals daily. Available in bottles of 100 and 1,000.

PARKE, DAVIS & COMPANY

DETROIT, MICHIGAN

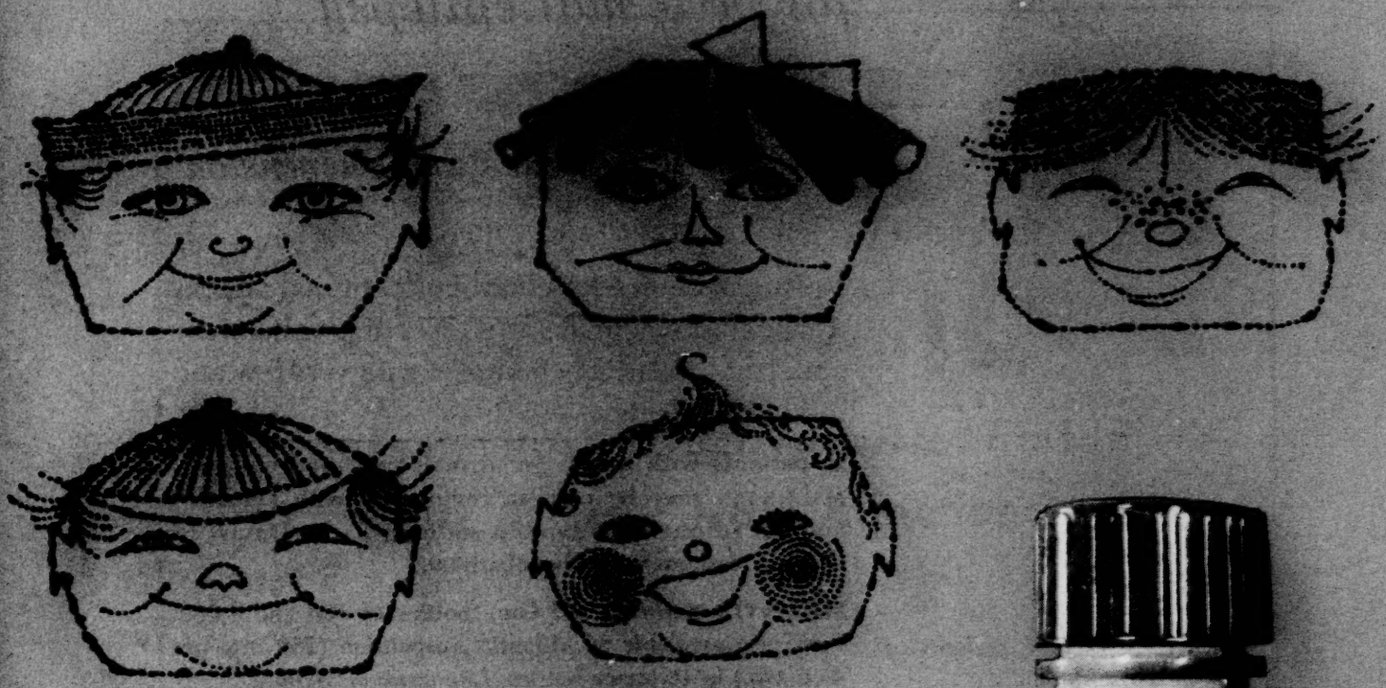


it's a rare child* who doesn't

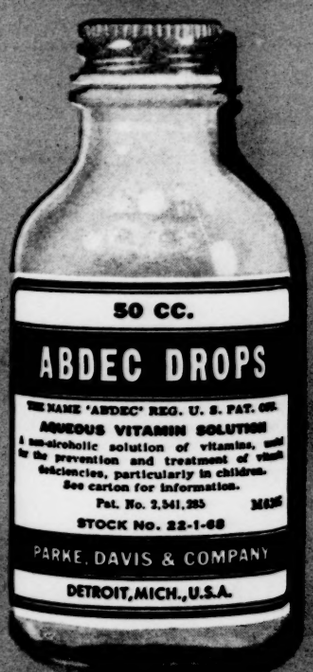
*

(and he's taking ABDEC Kapseals®)

PARKE, DAVIS & COMPANY - DETROIT, MICHIGAN



take to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ABDEC DROPS
THE NAME 'ABDEC' REG. U. S. PAT. OFF.
AQUEOUS VITAMIN SOLUTION
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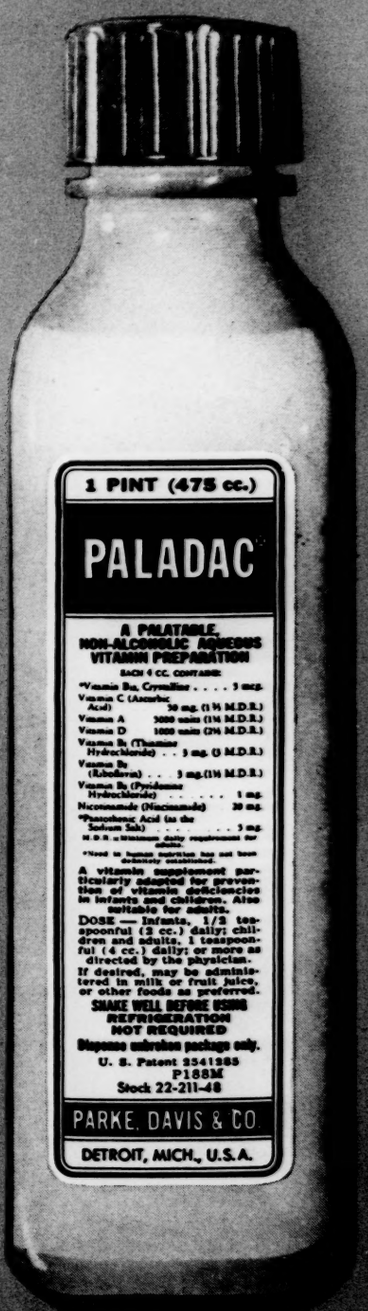
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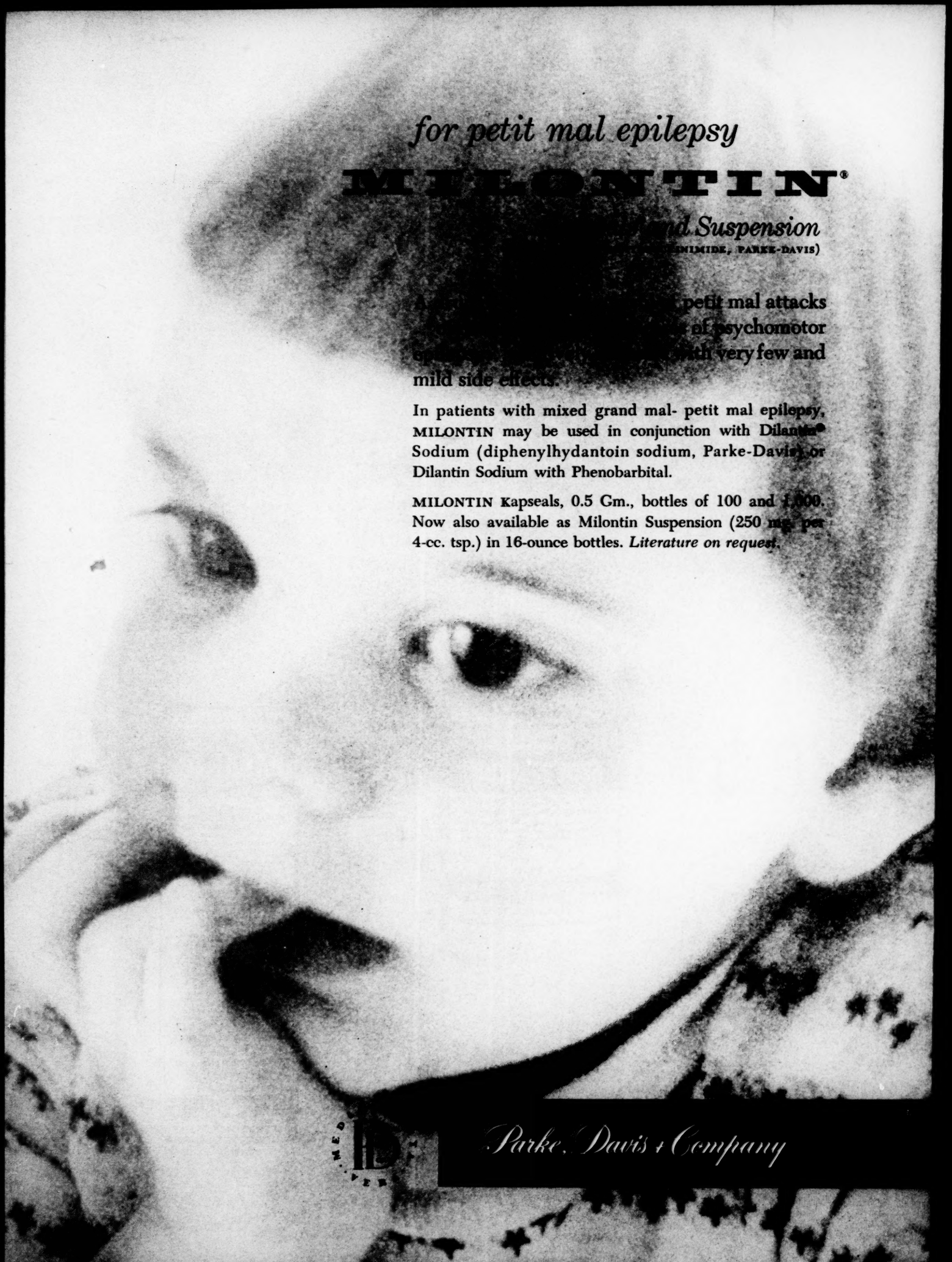
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(1) Payne, R. W.; Shetlar, M. R.; Farr, C. H.; Hellbaum, A. A., and Ishmael, W. K.: J. Lab. & Clin. Med. *45*:331, 1955. (2) Bunim, J. J.; Williams, R. R., and Black, R. L.: J. Chron. Dis. *1*:168, 1955. (3) Holbrook, W. P.: M. Clin. North America *39*:405, 1955.

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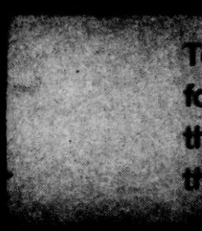

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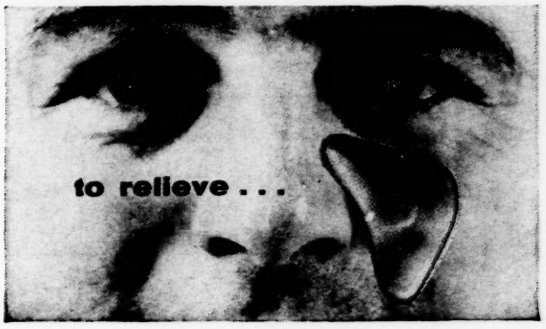

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1. Lazar, A. M., and Goldin, M.: Eye, Ear, Nose & Throat Monthly *32*:512, 1953.

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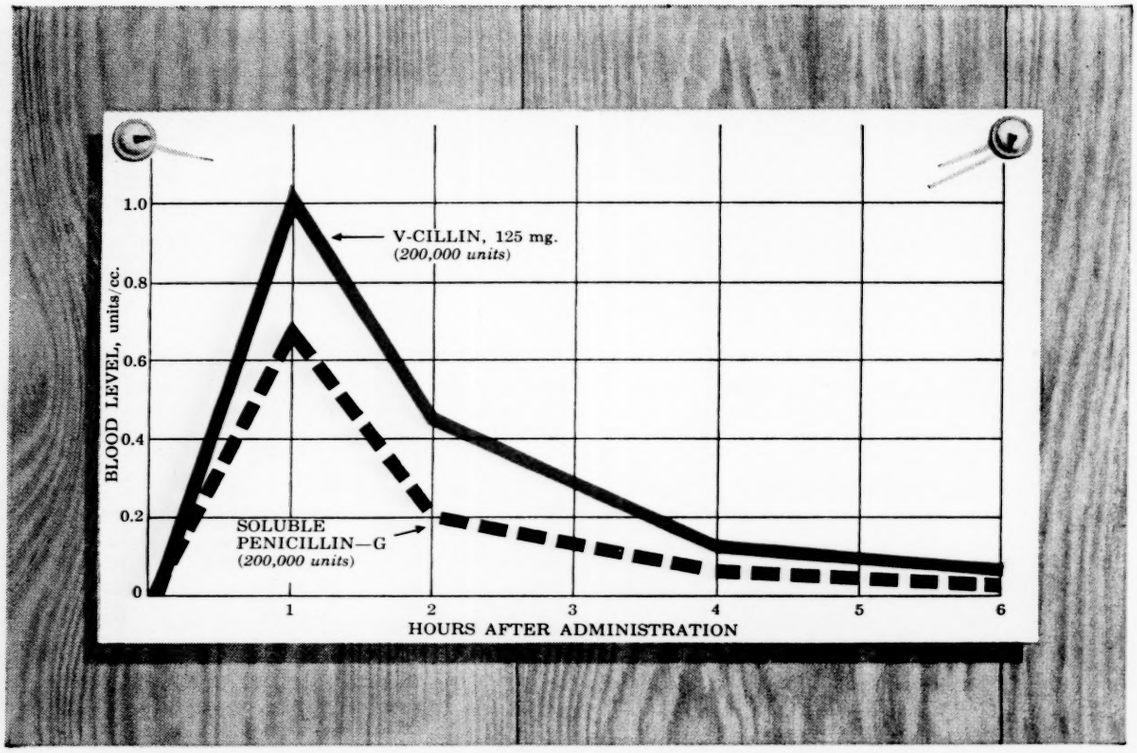

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The American Journal of Medicine

VOL. XX JANUARY, 1956 No. 1

Editorial

The Interrelation of Citrate and Calcium Metabolism

THE concept that most of the energy-producing reactions of the cell are linked to the citric acid cycle has been steadily strengthened since it was proposed by Krebs.[1] In this schema two carbon fragments derived from carbohydrate, fat and amino acids are condensed with oxaloacetate to form citrate through the mediation of coenzyme A. Citrate is constantly being synthesized and then converted through the successive steps of the cycle to oxaloacetate which again reacts to start the cycle anew. The levels of citrate in body fluids in the steady state should then be determined by the intracellular enzymatic processes governing the rates of citrate formation and conversion. Administered citrate rapidly disappears from the blood, as would be expected. Serum citrate levels are lowered by glucose and insulin injection[2] and also by administration of cortisone,[3] which may indicate a similar effect upon intracellular citrate. The conversion of citrate to cis-aconitic acid is competitively inhibited by fluorocitrate and fluoroacetate, leading to increased concentrations of citrate in tissues and also in blood.[4]

A long-recognized property of citrate is its effect upon the behavior of calcium in solution. An excess of citrate increases the solubility of poorly soluble calcium salts and antagonizes the physiologic effects of calcium ion. It was therefore postulated that an undissociated calcium citrate complex is formed, reducing the concentration of Ca^{++} ion. McLean and Hastings[5] were able to demonstrate this by their biological technic for measuring Ca^{++} ion activity and they suggested that calcium citrate dissociates in two stages as follows:

$$Ca_3Cit_2 = Ca(CaCit)_2 \rightleftarrows Ca^{++} + 2\ CaCit^-$$
$$CaCit^- \rightleftarrows Ca^{++} + Cit^{\equiv}$$

At the pH of the body fluids pK $CaCit^-$ was estimated to be 3.2 and this permits calculations of the Ca^{++} concentration in solutions of calcium salts and citric acid in this pH range. The average concentration of citrate in the serum of normal fasting subjects is 2.5 mg./100 cc., or 0.13 mM/L., and that of calcium 10 mg./100 cc., or 2.5 mM/L. In plasma not more than 0.5 mg. of calcium per 100 cc. could be sequestered by citrate so that the solubility of calcium in plasma or interstitial fluid would probably not be altered greatly by changes in the concentrations of citrate of the order of magnitude which have been observed.

A relationship between citrate and calcium metabolism has been found, however, which indicates that the metabolic reactions which influence the calcium economy of the body can affect the accumulation of citrate in tissues. This

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[2] NATELSON, S., PINCUS, J. B. and LUGOVOY, J. K. *J. Clin. Investigation*, 27: 446, 1948.

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may be an approach to the study of the specific cellular mechanisms involved. Studies of this relationship were stimulated by Dickens'[6] observation that bone is relatively rich in citrate which cannot be eluted from the bone by water. This citrate is thought to be held on the surface of the bone crystal by virtue of its property of complexing with calcium. A number of investigations have shown that vitamin D influences citrate metabolism, as measured by serum and tissue citrate concentrations and urinary excretion of citrate.[7–10] Serum citrate levels are reduced in rachitic infants and rats, and vitamin D increases the concentration of serum citrate and the urinary output of citrate. In the rachitic rat it has also been found that the concentration of citrate in bone as well as in other tissues, such as small intestine and kidney, is increased by vitamin D.

Reduced concentrations of serum citrate are also found in association with the hypocalcemia of hypoparathyroidism, and administration of parathyroid extract as well as vitamin D increases the serum citrate of the hypoparathyroid patient or parathyroidectomized dog.[11] Serum citrate concentrations moreover are found to be increased beyond the normal level in hypercalcemic states, whether the hypercalcemia be due to hypervitaminosis D, hyperparathyroidism or other causes. This might be interpreted as evidence that serum citrate levels are in some way determined by the concentrations of calcium in body fluids rather than being primarily influenced by vitamin D or parathyroid hormone. The only detailed studies which pertain to this point are those with vitamin D. The serum citrate levels in vitamin D deficient infants and rats are reduced irrespective of the levels of serum calcium so that before vitamin D is administered there is no correlation between serum citrate and calcium concentrations. Experiments with the rachitic rat have also shown that the serum citrate rises within twenty-four hours after physiologic doses of vitamin D, before any demonstrable change in serum calcium levels has occurred.[12] Findings such as these suggest that vitamin D does directly influence citrate metabolism and it may be that this also is true of other agents which affect the distribution of calcium. Whether or not this effect on citrate metabolism occurs primarily in the skeletal tissues is not known. It is of interest that the serum citrate levels are increased in patients with generalized Paget's disease who have markedly elevated serum alkaline phosphatase activities although their serum calcium levels are normal. *In vitro* studies have shown the presence of citrogenase and aconitase activity in bone tissue and it has been suggested that citrate accumulation in the skeleton could result from metabolic activity of bone cells.[13]

Intravenous injection of sodium citrate causes increased loss of calcium in the urine and demineralization of bone. Oral administration of sodium citrate to rachitic infants or rats, on the other hand, produces a drop both of serum calcium levels and of urinary calcium excretion along with healing of the rickets. It is likely that this apparent paradox is due to the fact that ingested citrate can sequester dietary calcium in the intestinal lumen and as a result intestinal absorption of phosphate is increased.[14] Deposition of calcium and phosphate in the rachitic cartilage results, then, because of increased availability of phosphate. The absorbed citrate is metabolized so rapidly that the levels of citrate in the systemic circulation are not persistently raised even by large amounts of ingested citrate.

The concentration of citrate in urine may have special physiologic significance since in this fluid, unlike plasma and interstitial fluid, the proportion of citrate to calcium may be such as to influence appreciably the state of the calcium. Urine containing 100 mg. calcium and 480 mg. citrate per liter is 2.5 mM with respect to each and at neutral pH, if no other factors were operating, more than half of the calcium would be complexed by citrate. Magnesium, however, also forms a complex with citrate so that an excess of magnesium would reduce the proportion of calcium in the undissociated state. Urine citrate has been included among the

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several factors which might increase the stability of calcium in urine and therefore function to prevent precipitation of calcium salts in the urinary tract.[15] Such suggestions have been made partly on the theoretic basis that the amount of citrate in urine can be sufficient to influence the ionization of calcium, and partly because of observations indicating a parallelism between urine citrate and calcium excretion. Such a parallelism is found, however, only under restricted conditions. Administration of alkalinizing salts for example results in a rise of urine citrate excretion but a decrease in urine calcium output, whereas acidifying salts cause a decrease in urine citrate and an increase of urine calcium. Citrate-to-calcium ratios were reported to be lower in patients with kidney stones than in normal subjects[16] but scant consideration has been given to the idea that this is a real difference. Since citrate disappears rapidly in infected urine owing to its utilization by bacteria it is difficult in these studies to separate the reduction of urine citrate due to some conjectural difference in metabolism or renal function from the effects of secondary infection. The role of urine citrate as an answer to the clinical problem of kidney stones may also have been quickly dismissed because the most effective method of elevating urine citrate output is by administration of the sodium or potassium salts of organic acids, with resultant alkalization of the urine. This is, of course, contrary to the accepted practice of trying to maintain an acid urine in patients with calcium stones. Estrogen-induced citraturia has also not had general clinical application.

A new aspect may have been added to this problem by the finding that diamox® administration reduces urinary citrate excretion of rats to negligible values. This observation led to examination of the kidneys of rats given diamox while on diets which produced high urine outputs of calcium.[17] Precipitation of calcium in the kidneys of the diamox-treated rats was demonstrated by chemical analysis as well as by histologic examination and the site of precipitation apparently depended upon the nature of the diet, particularly as it influenced urine phosphate. Control rats on the same diets excreted citrate in molar concentrations approaching those of calcium, and their kidneys showed no calcium precipitation. These experiments lend some support to the concept that urine citrate may be of importance in the prevention of precipitation of calcium salts in the urine and there may be renewed interest in examination of the mechanisms which determine citrate excretion in the urine not only in relation to the problem of renal calcinosis and stone formation but also with respect to other aspects of renal tubular function. The urinary excretion of citrate may vary without corresponding change in plasma citrate levels. This has been found in the diamox experiments as well as in studies of the variation of urine citrate with change in pH. The reduction of urine citrate excretion in states of potassium deficiency is also of interest. The output of citrate in urine is clearly modified by renal tubular activity and the relation of citrate metabolism to renal tubular function becomes of added significance in view of the special biochemical properties of this tricarboxylic acid.

HAROLD E. HARRISON, M.D.
Baltimore City Hospitals,
Baltimore, Md.

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Clinical Studies

Simmonds' Disease*

Evaluation of Certain Laboratory Tests Used in Diagnosis

PAUL P. VANARSDEL JR., M.D. *and* ROBERT H. WILLIAMS, M.D.

Seattle, Washington

In the majority of patients with pituitary deficiency the clinical findings should be sufficient to indicate the diagnosis. In a surprisingly large number of individuals, however, thyroid deficiency is so prominent that the primary disorder is not recognized unless certain laboratory tests are performed.

A procedure which would indicate definitely the presence of pituitary deficiency would be of inestimable value in the patient lacking strong clinical evidence of Simmonds' disease. Assays for thyrotropic and adrenotropic hormones in blood and urine have been developed[1–4] but so far have not proved of general clinical value. On the other hand, assays of human urine for follicle-stimulating hormone have been very useful; the quantities excreted have been determined for many age groups and disease states.[5–7]

The activity of thyrotropin and adrenotropin can be evaluated indirectly by measuring the respective end-organ functions. The insulin-tolerance test,[8] glucose-insulin tolerance test,[8,9] adrenal response to adrenotropin[10] and thyroid response to thyrotropin[11,12] are aids in the diagnosing of pituitary deficiency but for a variety of reasons none of these has been consistently satisfactory. The insulin tolerance test is potentially dangerous since it may cause death from hypoglycemia.[13,14] Even when specific precautions are taken the glycemic response to insulin, along with other tests for adrenal function, can be misleading. Thus Oelbaum has reported lack of hypoglycemia unresponsiveness in two of six patients with Sheehan's syndrome[15] and other cases have been recorded in which a deficiency of thyrotropin existed but adrenal function was apparently normal.[16,17] In the opposite situation, the frequent finding of laboratory evidence for adrenal hypoactivity in primary hypothyroidism makes adrenal function tests of limited value in diagnosing Simmonds' disease.[18–21] In this particular situation, to be sure, a period of thyroid replacement therapy can precede the final evaluation of adrenal function. This, however, is not usually a desirable procedure when pituitary deficiency is suspected.

A significant thyroid response to thyrotropin administration is a more satisfactory indication that pituitary deficiency is present. Yet certain cases of hypopituitarism exist in which the thyroid acinar cells are almost completely absent and no response occurs. In the large series reviewed by Sheehan and Summers fifteen of ninety-five autopsied subjects with chronic severe hypopituitarism had totally fibrosed thyroid glands.[13] A failure to respond to thyrotropin was found in one of the hypopituitary cases reported by Querido and Stanbury[11] and three of the six cases cited by Garrod and Gilliland.[21]

The inadequacy of the tests cited, used individually, prompted us to evaluate the relative significance of the urinary gonadotropin (FSH) assay,† which has been available for many years as an aid in the diagnosis of pituitary disorders.[22–24] In spite of its relative simplicity this determination is infrequently performed for the differential diagnosis of hypothyroid states. Since the urinary excretion of FSH is usually either markedly increased or markedly decreased in the presence of ovarian failure, and since a large number of cases of primary and

† For simplicity the term FSH will be used in the balance of this article although the assay method is not completely specific for this hormone.

* From the Department of Medicine, University of Washington School of Medicine, Seattle, Wash. Aided in part by grants from Smith, Kline and French Laboratories and the Atomic Energy Commisison.

secondary myxedema occur in this group,[22] the assay should contribute valuable information in a majority of patients with hypothyroidism.

CLINICAL MATERIAL AND METHODS

Almost all of the cases in this study were patients at the Boston City Hospital (made available to us by permission of Dr. William B. Castle), the Presbyterian Hospital, New York (with permission of Dr. Robert F. Loeb) or the King County Hospital, Seattle. Except for some of the patients at the Presbyterian Hospital, all patients were seen personally by one or both of the authors. All available patients with pituitary deficiency were examined and, if clinically or pathologically acceptable, were included in this study. The clinical diagnosis of pituitary disease was based on well established criteria[13] and depended on the coexistence of hypothyroidism (which was common to all our patients) with one or more of the following groups of findings: (1) A history of postpartum hemorrhage, followed by amenorrhea, absence of hot flushes, failure to lactate, and subsequent gonadal atrophy (Sheehan's syndrome). (2) Loss of skin pigment, loss of axillary and pubic hair, and appearance of hypogonadism. (3) Headache, visual field defects and roentgenographic evidence of sella turcica abnormalities. (4) Symptomatic adrenal deficiency in the absence of skin pigmentation. A definite rise in thyroidal I-131 uptake following thyrotropin administration and/or lack of clinical improvement after therapy with desiccated thyroid were considered to be evidence supporting the diagnosis of Simmonds' disease. Since the main purpose of this study was to evaluate the FSH assay, no case was included in this series in which a negative assay was the only adequate evidence for hypopituitarism.

In most of the cases studied at the Boston City Hospital urinary FSH assays were conducted on rats, by a modification of the method of Heller and Heller.[25] In the remainder, the urinary FSH was concentrated either by kaolin adsorption and alcohol precipitation[26] or by ultrafiltration and alcohol-ether precipitation;[27] assays were performed on twenty-one day old female mice.

Response to thyrotropin was gauged by observing the twenty-four-hour thyroidal I-131 uptake following three days of intramuscular administration of thyrotropin, 5 units twice daily (thytropar,® lot M2105, kindly supplied by Armour Laboratories, was used in patients studied at the King County Hospital). Serum cholesterol determinations were performed according to a modification of the method of Schoenheimer and Sperry.[28]

From the several hospitals and clinics a total of ninety-five patients was found suitable for case analysis. Those with pituitary hypofunction are listed in Table I. All patients from the King County Hospital with well documented primary myxedema were utilized, although only those from Boston City Hospital in whom gonadotropin assays had been performed were included, and none from Presbyterian Hospital were studied. The patients with primary myxedema are listed in Table II.

RESULTS

Sixty-two patients had thyroid hypofunction secondary to pituitary disease.* In twenty-three cases the diagnosis was confirmed by autopsy or craniotomy. Pituitary deficiency was caused by a proved or probable chromophobe adenoma in nineteen cases, by postpartum necrosis (Sheehan's syndrome) in fifteen cases, and by a craniopharyngioma in three cases. It was associated with acromegaly in two cases, and with an aneurysm of the internal carotid artery in one. Case 62 had multiple pulmonary lesions of unknown etiology in addition to pituitary insufficiency and diabetes insipidus, and may have had a granulomatous involvement of the pituitary or hypothalamus. In twenty-one patients the etiology was unknown.

The relationship of the urinary FSH excretion to the type of patient studied is shown in Figure 1. Two female patients with Simmonds' disease were found to excrete measurable amounts of FSH but the remainder had no demonstrable excretion of this substance. In only one postmenopausal woman with primary myxedema was the assay for urinary FSH negative. Assay was undertaken in only two males with primary myxedema; in one it was negative, in the other, strongly positive.

The distribution of the serum cholesterol values in the two groups is shown in Figure 2. The dotted line represents the observations in a

* Some of these cases have been included in previous studies: Cases 3, 4, 29, 34, and 35 by Williams and Whittenberger;[23] Cases 3, 4, 8, 29, 34, and 35 by Williams et al.;[29] Cases 1 to 5, 8, 25 to 28, 30, and 32 to 35 by Daughaday, Williams and Daland;[30] Cases 3, 31, and 35 by Klinefelter, Albright and Griswold;[24] Case 37 by Werner;[31] and Case 43 by Knowlton et al.[32]

total of forty-six patients with myxedema reported by Hurxthal[33] and by Gildea, Man and Peters.[34] Almost one-half of our patients with pituitariprival hypothyroidism had total serum cholesterol levels below 200 mg. per 100 ml. whereas all subjects with primary myxedema had values above 200. However, it should also be noted that an occasional patient with Simmonds' disease had a markedly elevated serum cholesterol value.

excretion was low in twenty-seven of twenty-eight patients with secondary hypothyroidism (Table I) but was depressed also in the three patients with primary myxedema in whom it was measured. The insulin tolerance test was sometimes normal in the hypopituitary group and, more significantly, hypoglycemia unresponsiveness was found in two patients with primary myxedema; the fall in blood sugar was not significantly delayed.

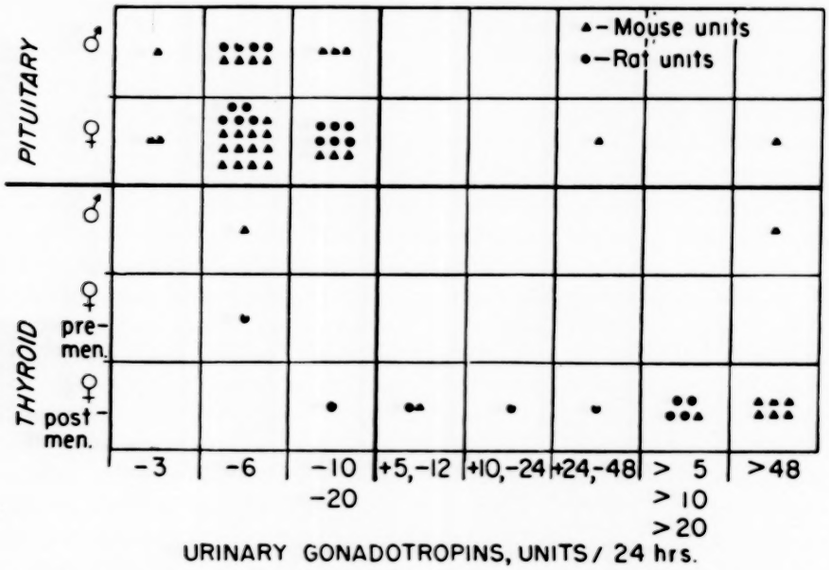

FIG. 1. Results on gonadotropin assays in sixty-two patients: (–) indicates lack of response at that particular dilution; (+) indicates presence of a response; (>) indicates positive response at that dilution, when negative responses at higher dilutions were not obtained.

The twenty-four-hour uptake of radioactive iodide by the thyroid gland was determined in eighteen patients with pituitary hypothyroidism. Whereas the average (11.7 per cent of the administered dose) was in the hypothyroid range, several were normal. A significant rise following thyrotropin administration occurred in six of the seven patients tested. In an eighth patient, case 3, with autopsy-proved hypopituitarism, thyrotropin administration did not raise the basal metabolic rate.* The uptake of I-131 was determined in only three patients with primary myxedema.

In forty-seven hypopituitary patients the basal metabolic rate averaged −30 per cent; in twenty-one patients with primary myxedema the average was −27 per cent. (Tables I and II.)

The twenty-four-hour urinary 17-ketosteroid

* Details of the autopsy findings have been previously reported by Williams et al.[29]

Of the sixty-two patients with anterior pituitary hypofunction, only Cases 24 and 62 had diabetes insipidus.

DISCUSSION

The original purpose of this study was to evaluate the FSH assay in the differentiation of primary myxedema and Simmonds' disease. It is of comparatively little value for such differential diagnosis in the male and in the premenopausal female (except for the special situations to be discussed) because of the relative insensitivity of the assay method, coupled with the tendency for the pituitary secretion of gonadotropins to be depressed to an undetectable level in primary myxedema. On the other hand, the level of urinary FSH in postmenopausal women is usually much higher than in premenopausal subjects, even in the presence of primary myxedema, although the latter may be

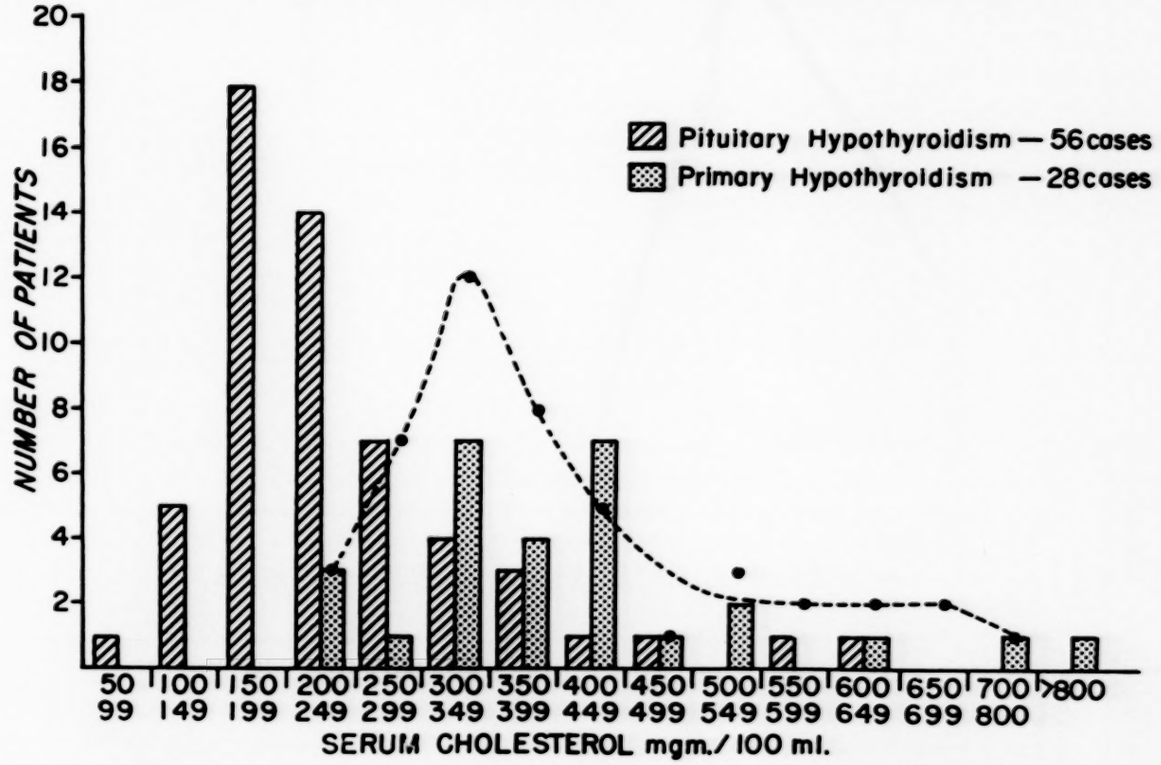

FIG. 2. Distribution of serum cholesterol values in eighty-four patients with hypothyroidism. The broken line indicates the distribution of serum cholesterol in forty-six other patients with myxedema (see text).

associated with less hypergonadotropism. In these individuals, and also in younger women with hypothyroidism and amenorrhea, in whom menopausal symptoms have not occurred, the absence of FSH excretion makes pituitary hypothyroidism almost a certainty. An exception to this rule might be Case 65, a white woman aged sixty-eight with obesity and long-standing myxedema, who had a FSH assay which was negative at 10 rat units, and who showed hypoglycemia unresponsiveness following insulin administration. Postmortem examination ultimately revealed an atrophic thyroid gland with an intact pituitary. It is possible however that the improved assays used recently might have demonstrated FSH excretion at a lower level.

In two patients with hypopituitarism FSH was excreted in measurable amounts: In Case 11, the diagnosis was proved at autopsy, and in Case 61 it was based on clinical findings plus a definite rise in thyroidal I-131 uptake from 6 to 21 per cent following thyrotropin administration. These individuals apparently had partial pituitary deficiency with relative preservation of gonadotropic function, a situation which is being recognized with increasing frequency.[16,17,35,36] One other patient (Case 29) with a positive urinary FSH assay has been reported in detail elsewhere[29] and was entering an unexplained, spontaneous remission when this determination was made. A previous assay, performed when symptoms were unequivocal, had been negative.

From these studies it is our conclusion that the urinary FSH determination is most useful in evaluating the myxedema or hypothyroidism of postmenopausal women. Thus far we have found that the FSH was always excreted in sufficient amounts in primary myxedema to be assayed at 6 mouse units, and was usually detected at higher dilutions. Because of the occasional appearance of partial pituitary deficiencies, the presence of FSH excretion does not rule out pituitary disease entirely, especially if other suggestive findings are present. In uncomplicated myxedema, however, the finding of FSH in the urine is strong evidence for primary thyroid deficiency; this is just as true if, fortuitously, this finding occurs in males and menstruating females.

The increasing recognition of cases of partial pituitary deficiency makes it advisable to

TABLE I

CASES OF HYPOTHYROIDISM CAUSED BY PITUITARY DEFICIENCY

Case	Hospital	Number	Age and Sex	Etiology	Sella Turcica	Basal Metabolic Rate (%)	Serum Protein-bound Iodine (γ/100 ml.)	Serum Total Cholesterol (mg./100 ml.)	I-131 Uptake*	FSH†	Urinary 17-Keto-steroids (mg./24 hr.)	ITT‡
					I. Proved at Autopsy or Craniotomy							
1	BCH	1184073	68, F	Unknown	.....	.....	...	230	.....	Neg. 5mu	....	.....
2	BCH	1182009	61, F	Unknown	.....	.....	...	181	.....	Neg. 5ru	1.4	.....
3	BCH	1047166	42, F	Sheehan's syndrome	.....	−47 TSH-negative	...	262	.....	Neg. 3 mu	0.4 1.9	.....
4	BCH	764228	47, F	Unknown	.....	−26	...	137	.....	Neg. 10ru	....	.....
5	BCH	1217495	57, M	Chromophobe tumor	.....	−40	...	218	.....	.....	1.24	.....
6	BCH	1320591	48, F	Unknown	.....	.....	...	198	0% TSH-positive	Neg. 3mu	0.4	.....
7	BCH	1106763	44, F	Chromophobe tumor	.....	.....	...	...	.....	Neg. 5ru	....	.....
8	BCH	111478	42, M	Chromophobe tumor	.....	.....	...	...	.....	Neg. 5ru	0.0	.....
9	PH	578425	32, F	Sheehan's syndrome	.....	−38	...	202	.....	Neg. 5mu	....	.....
10	PH	601520	34, F	Sheehan's syndrome	.....	−33	...	229	.....	Neg. 5mu	1.1	.....
11	PH	719087	49, F	Sheehan's syndrome	.....	−26	...	164	.....	Pos. 20mu	1.8	.....
12	PH	461599	36, F	Unknown	.....	−32	...	180	.....	.....	....	.....
13	PH	836798	67, M	Chromophobe tumor	.....	−37	...	250	.....	.....	....	.....
14	PH	118366	37, F	Chromophobe tumor	.....	−36	...	495	4%	Neg. 5mu	0.17	.....
15	PH	543752	38, M	Chromophobe tumor	.....	−34	...	194	.....	.....	....	.....
16	PH	756386	50, M	Chromophobe tumor	.....	−31	...	340	15% TSH-positive	.....	0.34	.....
17	PH	813022	36, M	Craniopharyngioma	.....	−38	...	180	.....	.....	1.3	.....
18	PH	497847	20, F	Carotid aneurysm	.....	−28	...	255	.....	.....	0.5	.....
19	KCH	78597	27, F	Sheehan's syndrome	.....	−20	...	148	.....	.....	....	.....
20	KCH	3127	76, M	Chromophobe tumor	.....	−25	...	291	20%	.....	2.2	.....
21	KCH	251210	70, F	Unknown	.....	.....	...	174	.....	.....	....	.....
22	KCH	144272	61, M	Craniopharyngioma	.....	−31	...	...	.....	Neg. 6mu	1.1	.....
23	SVAH§	7832	37, M	Chromophobe tumor	.....	−34	...	192	.....	.....	....	.....
					II. Clinically Typical Cases							
24	EMH**	317789	20, F	Unknown	N	−34	...	158	.....	Neg. 5ru	....	Equivocal
25	BCH	1229539	69, M	Unknown	N	.....	...	162	.....	Neg. 5ru	....	Pos.
26	BCH	1212801	53, F	Unknown	Calcified	.....	...	200	.....	Neg. 5ru	....	Pos.
27	EMH	321526	61, F	Unknown	N	.....	...	115	.....	Neg. 10ru	3.3	Pos.
28	BCH	1183114	48, F	Unknown	N	−26	...	150	.....	Neg. 5ru	....	Pos.
29	BCH	1054056 1058888	65, F	Unknown	N	−33	...	129	.....	Neg. 20ru	1.4	.....
30	TH††	2278	21, F	Sheehan's syndrome	.....	−31	...	...	.....	Neg. 10ru	4.4	Neg.
31	MGH‡‡	177833	33, F	Sheehan's syndrome	N	−40	...	112	.....	Neg. 6mu	0.5	Pos.
32	BCH	1200750	43, M	?Chromophobe tumor	Large	.....	...	166	.....	Neg. 5ru	....	Neg.
33	BCH	782531	59, M	?Chromophobe tumor	Thin floor	.....	...	200	.....	Neg. 5ru	....	Pos.
34	BCH	1024858 1048285	38, F	?Chromophobe tumor	Large	−30	...	...	.....	Neg. 10ru	0.4	Pos.
35	BCH	1035901 1047262	46, M	?Chromophobe tumor	Large	−36	...	...	.....	Neg. 6.6 mu	0.8	Pos.
36	PH	082459	59, M	Unknown	N	−17	...	165	11%	.....	2.7	.....

TABLE 1 (*Continued*)

Case	Hospital	Number	Age and Sex	Etiology	Sella Turcica	Basal Metabolic Rate (%)	Serum Protein-bound Iodine (γ/100 ml.)	Serum Total Cholesterol (mg./100 ml.)	I-131 Uptake*	FSH†	Urinary 17-Keto-steroids (mg./24 hr.)	ITT‡
37	PH	088124	58, M	Unknown	N	−36	...	184	15% TSH-positive	.....	....	.....
38	PH	719910	51, F	Sheehan's syndrome	N	−28	...	163	.....	Neg. 10ru	1.1	.....
39	PH	784431	60, F	Sheehan's syndrome	N	−37	...	293	.....	.....	2.5	Pos.
40	PH	134794	55, F	Sheehan's syndrome	N	−23	...	434	16%	Neg. 20mu	4.1	.....
41	PH	956118	33, F	Sheehan's syndrome	N	−30	...	211	22%	Neg. 10mu	....	.....
42	PH	117393	44, F	Sheehan's syndrome	N	−23	...	395	17%	Neg. 10mu	1.25	.....
43	PH	832794	51, F	Sheehan's syndrome	.....	−41	...	587	2%	.....	1.0	.....
44	PH	783549	67, M	?Chromophobe tumor	Large	−19	...	232	.....	.....	3.5	.....
45	PH	953236	54, M	?Chromophobe tumor	Large	−32	...	286	.....	Neg. 5mu	2.8	.....
46	PH	978350	44, F	?Chromophobe tumor	Large	−16	...	251	25%	.....	....	.....
47	PH	995768	59, M	?Chromophobe tumor	Large	−30	...	238	.....	.....	....	.....
48	PH	896208	60, M	?Chromophobe tumor	Large	−23	...	228	.....	.....	...	.....
49	PH	133798	60, M	?Chromophobe tumor	Large	−31	...	381	30%	Neg. 20mu	4.2	.....
50	PH	853821	28, M	Craniopharyngioma	Large	−17	...	242	.....	Neg. 5mu	0.3	.....
51	PH	879915	39, M	Acromegaly	Large	−37	...	178	.....	Neg. 10mu	1.9	.....
52	PH	754773	34, F	Acromegaly	.....	−46	...	195	24%	Neg. 10mu	4.8	.....
53	KCH	32483	40, F	Sheehan's syndrome	N	−40	...	370	.....	Neg. 6mu	1.0	.....
54	Private		35, F	Sheehan's syndrome	N	−17	...	310	.....	Neg. 6mu	0.8	.....
55	KCH	189146	75, F	Unknown	N	.....	...	600	.....	Neg. 6mu	9.4	.....
56	KCH	214568	65, F	Unknown	N	.....	...	232	.....	Neg. 6mu	....	.....
57	KCH	315423	74, F	Unknown	N	.....	...	91	1% TSH-positive	Neg. 6mu	0.4	.....
58	KCH	297172	76, F	Unknown	N	−30	1.2	320	5% TSH-negative	Neg. 6mu	1.4	.....
59	KCH	318339	68, F	Unknown	Large	−1	1.1	183	0% TSH-positive	Neg. 6mu	....	.....
60	KCH	317962	51, F	Unknown	N	−25	...	213	2%	Neg. 6mu	2.3	.....
61	KCH	439	54, F	Unknown	N	.....	1.7	303	6% TSH-positive	Pos. 48mu	....	.....
62	KCH	304676	47, M	?Granuloma	N	−20	...	224	.....	Neg. 3mu	3.1	.....

* Twenty-four hour-thyroidal uptake, per cent of administered dose.

† Expressed as units at which the assay was performed on a twenty-four-hour urine specimen.

‡ Insulin tolerance test: considered "positive" when the blood sugar was still depressed ninety minutes after intravenous insulin administrations. When a normal ("neg.") response occurred with 0.03 unit of insulin/kg. of body weight, test was repeated using 0.1 units insulin/kg.

§ Seattle Veterans Administration Hospital.

** Evans Memorial Hospital, Boston.

†† Thorndyke Memorial Laboratory, Boston.

‡‡ Massachusetts General Hospital, Boston.

evaluate the function of all three tropic hormones in an occasional patient. The FSH determination can be valuable in all adults where such a complete survey is indicated. When the level is found to be subnormal or negative in a male or menstruating female, the assay can then be repeated after several weeks of watchful thyroid replacement therapy, which should be associated with an increase in gonadotropin production in primary but not secondary

TABLE II

CASES OF HYPOTHYROIDISM CAUSED BY PRIMARY THYROID DEFICIENCY

Case	Hospital	Number	Age and Sex	Basal Metabolic (%)	Serum Protein-bound Iodine (γ/100 ml.)	Serum Total Cholesterol (mg./100 ml.)	I-131 Uptake	FSH	Urinary 17-Ketosteroids (mg./24 hr.)	ITT
I. Proved at Autopsy, or Due to Thyroid Ablation										
63	BCH	1068513	47, F	....	...	243	.....	Pos. 5ru	...	.....
64	BCH	1157414	39, F	−32	...	286	.....	Neg. 5ru	1.4	Neg.
65	BCH	1129686	68, F	−44	...	500	.....	Neg. 5ru	...	Pos.
66	KCH	250228	54, F	−26	...	618	.....	.....	...	.....
67	KCH	289173	55, F	....	1.3	272	.....	.....	....	.....
68	KCH	295187	55, F	−7	...	310	.....	.....	...	.....
69	KCH	247385	59, F	−28	...	350	.....	.....	....	.....
70	KCH	94621	70, F	....	...	310	.....	.....	...	.....
71	KCH	243666	60, M	−16	...	435	.....	.....	...	.....
72	KCH	206162	55, M	....	...	527	.....	.....	...	.....
II. Clinically Typical Cases										
73	BCH	1099003	54, F	−33	...	357	.....	Pos. 10ru	...	.....
74	BCH	1217768	72, F	−40	...	227	.....	Pos. 5, Neg. 20ru	...	.....
75	BCH	1091920	50, F	−44	...	...	.....	Pos. 10ru	...	Pos.
76	BCH	1058030	68, F	−24	3.0	...	.....	Pos. 20ru	2.1	.....
77	BCH	1195358	65, F	....	...	...	.....	Pos. 20ru	...	.....
78	BCH	1059295	66, F	−34	...	...	.....	Pos. 10, Neg. 20ru	...	Pos.
79	KCH	122599	66, F	−24	...	400	.....	Pos. 6mu, Neg. 96mu	...	.....
80	KCH	304002	53, F	....	...	430	.....	.....	...	.....
81	KCH	11674	65, F	−12	...	339	.....	.....	....	.....
82	KCH	282981	68, F	....	...	408	.....	.....	...	.....
83	KCH	263100	71, F	−21	3.2	424	.....	Pos. 48mu	...	.....
84	KCH	270885	58, F	−30	...	806	.....	Pos. 48mu	...	.....
85	KCH	277441	74, F	....	...	480	.....	.....	...	.....
86	KCH	228622	63, F	−26	...	212	15% TSH neg.	Pos. 48 mu	...	.....
87	KCH	183268	63, F	−35	...	400	.....	.....	...	.....
88	KCH	142093	48, F	−11	1.2	336	.....	.....	...	.....
89	KCH	315808	49, F	....	1.4	449	4% TSH neg.	Pos. 48mu	...	.....
90	KCH	132568	55, F	....	2.5	355	.....	Pos. 6, Neg. 12mu	...	.....
91	KCH	322345	76, F	....	2.2	368	3%	Pos. 48mu	...	.....
92	KCH	86035	56, F	−17	...	740	.....	Pos. 96mu	...	.....
93	KCH	302544	68, M	....	...	343	.....	Neg. 6mu	1.7	.....
94	KCH	238941	64, M	−27	...	300	.....	.....	...	.....
95	KCH	297390	59, M	−34	...	332	.....	Pos. 48mu	...	.....

myxedema. This phenomenon was first reported by Statland and Lerman.[19] Beierwaltes and his associates have noted a consistent rise in FSH excretion in the male and premenopausal female during thyroxin therapy[37] and Solomon has recently observed that a significant rise in urinary gonadotropin excretion occurred in ten male patients with primary myxedema.[38]

The total serum cholesterol is of less value than the gonadotropic hormone assay in the differential diagnosis of primary myxedema and Simmonds' disease but may be of some help if very low. Why a low cholesterol level is so frequent in secondary hypothyroidism is not clear. The higher average protein-bound iodine levels and thyroidal I-131 uptakes in the pituitary group (*v. seq.*) suggest that partial preservation of thyroid function in some patients might explain the lower cholesterol values. There is considerable evidence that this actually occurs. Entenman, Chaikoff and Reichert demonstrated that very little rise in serum lipids occurred in dogs following hypophysectomy but when the thyroid gland was subsequently removed from the same animals an abrupt and consistent rise in all blood lipids occurred.[39] Peters and Man have observed that thyroid replacement therapy could be given to patients maintained at myxedematous levels with thiouracil, in doses sufficient to lower the serum cholesterol to normal without producing clinical improvement or elevating the basal metabolic rate.[40] In the hypophysectomized rat thyroid activity is still present, although functioning at about 10 per cent of its normal capacity.[41]

We found that the twenty-four-hour thyroid I-131 concentration was sometimes appreciable in the hypopituitary individuals, being above 15 per cent in eight of the eighteen patients studied. Others[12,42] have observed that the I-131 uptake is somewhat higher in secondary than in primary hypothyroidism. Skanse has reported serum protein-bound iodine levels to be higher in secondary than in primary myxedema[43] and Peters et al. showed that the low basal metabolic rate in pituitary hypofunction correlated poorly with the serum "hormonal" iodine in some patients.[44]

Although there is evidence that some thyroidal function persists in certain cases of hypopituitarism, there is no significant difference in the average basal metabolic rate between patients with Simmonds' disease and those with primary myxedema, either in our series or in those of others.[45] The low basal metabolic rate found in Simmonds' disease may be due in part to inactivity of another target organ, most likely the adrenal gland. Adrenal cortical deficiency alone may be associated with a moderately low basal metabolic rate[46] which, according to one investigator, can be raised to normal by the administration of sodium chloride.[47]

A positive response to exogenous thyrotropin has been of great value in determining the presence of thyrotropin deficiency. Since our experience was limited to a small percentage of our patients, analyses of the findings of others in the use of thyrotropin are added to our own in Table III.

The lack of response in a large percentage of those patients with hypopituitarism studied during earlier years does not necessarily indicate the presence of an unresponsive thyroid gland. The hormone preparations were crude, and had to be given for comparatively long periods of time before any elevation in the basal metabolic rate could occur. This was an ideal situation for the appearance of antihormones, first demonstrated by Wachstein,[49] and such a mechanism, in all likelihood, completely prevented a metabolic response in some of the patients. Apparently, antihormone effect is now no problem since a response to TSH invariably occurs in known euthyroid persons.[42,59,63] Thus the lack of response to TSH noted in six of the patients with hypopituitarism more recently studied is a good indication that their glands were completely atrophied. However, Perloff and colleagues have considered the TSH test to be more absolute, and reported two cases in which they changed the clinical diagnosis of hypopituitarism to that of primary myxedema on the basis of the TSH response alone.[12] These patients are indicated in parentheses in Table III.

It is difficult to understand how a thyroid response to thyrotropin could occur in spontaneous primary myxedema, although this phenomenon has been reported by Wachstein and by Querido and Stanbury. (Table III.) Since the diagnosis was not anatomically proved, these patients may have had thyrotropin deficiency, a possibility which the latter authors admit.

The group studied by Kurland is unique in that myxedema was induced by I-131 administration in order to alleviate their intractable heart disease. Here the presence of residual thyroid tissue was demonstrated by TSH stimulation. Similarly, TSH produced a small

TABLE III
RESPONSES TO TSH IN HYPOTHYROIDISM

Group	Year	Test	Primary Myxedema		Hypopituitarism	
			Present	Absent	Present	Absent
Schittenhelm, Eisler[48]	1932	BMR	0	1	.....	.....
Wachstein[49]	1934	BMR	1	0	0	1
Lederer[50]	1935	BMR	...	...	2	0
Starr[51]	1935	BMR	0	3	1	0
Thompson et al.[52]	1936	BMR	0	4	7	6
Bulger, Barr[53]	1936	BMR	...	...	1	2
Scowen[54]	1937	BMR	0	6	3	0
Spence, Witts[55]	1939	BMR	...	...	2	0
Sharpey-Schafer, Schrire[56]	1939	BMR	0	4	.....	.....
Schrire[57]	1949	BMR	0	1	3	0
Werner et al.[58]	1950	PBI,RAI	0	6	3	0
Querido, Stanbury[11]	1950	PBI,RAI	1	5	2	1
Perloff et al.[12,59]	1951 1954	RAI	1	26	12	0(2)
Skanse[43]	1953	PBI,RAI	0	10	8	0
Shuman[16]	1953	RAI	...	...	1	0
Jefferies[60]	1953	PBI,RAI	1*	11	1	0
Kurland et al.[61]	1954	RAI	2	1	.....	.....
Koepf[62]	1954	RAI	...	...	1(3)	0
Garrod, Gilliland[21]	1954	RAI	0	9	3	3
Sampson[17]	1954	PBI,RAI,BMR	...	...	1	0
Bishopric[42]	1955	RAI	0	12	5	1
VanArsdel, Williams	1955	RAI	0	4†	6	1
		BMR	...	...	.....	.....
		Totals				
		BMR	1	19	19	10
		RAI	5	84	43	6

PBI = protein-bound iodine.
RAI = radioactive iodine uptake.
* This patient was a goitrous cretin in whom the I-131 trapping mechanism was presumably intact.
† Two of these patients were seen after the main portion of our study was completed and are not included in Table II.

increase in the thyroidal I-131 uptake of five of the twenty-seven patients reported by Schneeberg et al.[59] These patients all had postoperative myxedema, in which presumably small amounts of tissue were left *in situ;* the change in I-131 uptake was not great enough to be classified as positive according to the criteria of Bishopric et al.,[42] except in one person. By these same criteria, only one of Koepf's three cases of Simmonds' disease can be considered to have had a significant response to TSH; the other two are thus indicated in parentheses in Table III.

The evidence presented by other workers[18–21] indicating that primary myxedema may be associated with adrenal insufficiency is supported by the finding of low urinary 17-ketosteroid excretion and hypoglycemia unresponsiveness in a few of our patients with that diagnosis. (Table II.) However, signs of marked adrenal deficiency, such as the occurrence of spontaneous hypoglycemia and specific electrolyte imbalances, are not found in primary myxedema. These signs indicate the presence of pituitary deficiency in the patient with hypometabolism, except in that rare instance in which primary thyroid and adrenal failure coexist.[64]

SUMMARY

In sixty-two patients with Simmonds' disease and thirty-three with primary hypothyroidism, various tests in differential diagnosis were

evaluated. In postmenopausal females the FSH assay was one of the best tests but it was of little value in males and in premenopausal females until after adequate thyroid replacement therapy. As reported by others, a rise in the I-131 uptake by the thyroid gland, following thyrotropin administration, was good evidence for secondary hypothyroidism; adrenal function studies were less helpful. Two patients apparently had incomplete pituitary deficiencies.

The distribution of serum cholesterol levels was compared in the two groups, as were the thyroidal I-131 uptakes and basal metabolic rates. In agreement with the findings of others, some patients with Simmonds' disease have laboratory evidence indicating that thyroid function is partially preserved, although this may not be apparent from the clinical state or basal metabolic rate.

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The Diagnostic Value of Plasma and Urinary 17-Hydroxycorticosteroid Determinations in Cushing's Syndrome*

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THE physical and metabolic abnormalities which constitute Cushing's syndrome are striking and easily recognized in their fully developed form. However, clinical differentiation of the benign (adenoma, hyperplasia) from the malignant (carcinoma) types of adrenocortical lesions which may result in hyperfunction is more difficult. Now that bilateral subtotal adrenalectomy and resection of adenomas have become safe and often curative procedures in many patients,[1] accurate diagnosis and evaluation of adrenocortical hyperfunction has become more important.

With the recent development of methods for estimating adrenocortical function it has become possible to study the hormonal imbalance quantitatively. Particular attention has been directed toward the urinary excretion of 17-ketosteroids in patients with Cushing's syndrome, especially in an effort to differentiate adrenocortical hyperplasia from neoplasm.[2,3] There is as yet little information concerning the metabolism of the 17-hydroxycorticosteroids in this disorder.

It is the purpose of this communication to report the alterations in plasma and urinary 17-OH-corticosteroids (17-OH-CS) and urinary 17-ketosteroids (17-KS) observed in three patients with Cushing's syndrome, two of whom have undergone surgery. The first patient had adrenocortical carcinoma, the second adenoma, and the third bilateral hyperplasia. It is believed that the response of the plasma 17-OH-CS to ACTH administered intravenously may be helpful in distinguishing cases with benign lesions of the adrenal cortex from those with malignant tumors.

METHODS

The subjects were studied on a metabolic ward. Urine was collected over a twenty-four-hour period (8 A. M. to 8 A. M.) and kept in a refrigerator without preservative until the collection period was terminated; an aliquot was then frozen and later analyzed for 17-OH-CS, 17-KS and creatinine. Relative constancy of creatinine excretion was accepted as evidence of adequate collection technics.

Blood samples for plasma 17-OH-CS determinations were drawn between 8 A. M. and 9 A. M., at a time when the subject was fasting. Thirty milliliters of blood were placed in a tube containing 4 mg. of heparin and immediately centrifuged. The plasma was separated and stored in a deep freeze until the time of analysis.

ACTH tests were performed by infusing intravenously 25 I.U. of corticotropin, diluted in 1,000 ml. of 5 per cent dextrose in water, over a six-hour period.[4] To avoid the effect of spontaneous diurnal variation in plasma 17-OH-CS levels, the infusion was always started between 8 A. M. and 9 A. M. Blood samples for 17-OH-CS determinations were drawn before the infusion was begun and at two-hour intervals thereafter.

Spontaneous diurnal variation of plasma 17-OH-CS was studied by drawing blood samples at four-hour intervals during the day; on these days the subjects were allowed their usual activities and food.

Analytic Methods. The following methods were used:

1. Creatinine was determined by the method of Peters.[5]

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2. Plasma 17-OH-CS were measured by the method of Nelson and Samuels[6] as modified by Eik-Nes.[7]

3. For determination of urinary 17-KS, a modification of the method of Callow[8] was used. To a 15 ml. aliquot of a twenty-four hour urine specimen, 1.5 ml. of 63 per cent H_2SO_4 were

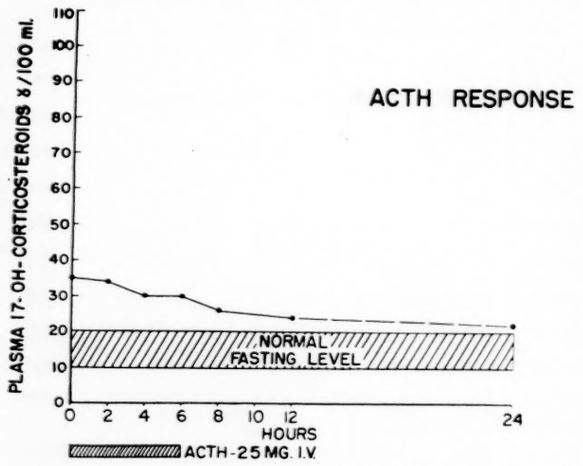

FIG. 1. Case 1, V. H. (adrenocortical carcinoma). The response of the plasma 17-OH-CS level to a standard infusion of ACTH is depicted.

added. The sample was then placed on a hot plate and refluxed for twenty-five minutes. The hydrolysate was rapidly cooled and then extracted three times with 15 ml. of ether. The ether extract was washed twice with 1 N NaOH and twice with redistilled water, each washing being made with 10 ml. The ether extract was evaporated to dryness and the residue was taken in 1 ml. of ethanol, 0.2 ml. of the extract being used for the Zimmerman reaction and 0.2 ml. for the sample blank. After incubation at 37°C. for ninety minutes the solutions were read on a Beckman model DU spectrophotometer at wave lengths of 440, 520 and 600 mμ. The optical density of the sample was compared with that of a known amount of dehydroepiandrosterone.

4. Urinary 17-OH-CS was measured by two different methods: (a) 17-OH-CS (conjugated as glucuronides) by means of hydrolysis with β-glucuronidase (Glenn and Nelson[9]). (b) 17-OH-CS (free and conjugated) by means of a modification[10] of the Reddy[11] method of serial extractions with chloroform and butanol at pH 1.0. Unless otherwise specified, urinary 17-OH-CS excretion was determined by the β-glucuronidase method. In our laboratory this method yields twenty-four-hour excretion values about twice those obtained by the Reddy technic.

5. The other biochemical determinations were carried out by conventional methods.

CASE REPORTS

Adrenocortical Carcinoma

CASE 1. V. H., a twenty-nine year old white male mechanic, was admitted to the hospital on January 19, 1953. Two months before admission he had noticed the onset of shortness of breath which gradually progressed. During this two-month period he had also noticed mild ankle edema and occasional attacks of nocturnal dyspnea. His face became puffy and took on a "sun-burned" appearance. An increase in abdominal girth, cutaneous acneform lesions and a decrease in libido were also described by the patient. Two weeks before admission he began to experience an aching intermittent right upper quadrant pain, nocturia and frequency of urination.

Physical examination revealed a young man with "moon" facies and a marked degree of rubor, especially over the neck and face. There was a prominent cervicodorsal fat pad. The blood pressure was 142/100. The skin over the chest, abdomen and extremities was slightly cyanotic and had a mottled appearance. Acne was evident over the face, neck and trunk. Physical examination of the chest was normal except for slight dullness to percussion at the right lung base. The abdomen was tense and protuberant and shifting dullness was detected. No abdominal striations were present. The spleen tip was felt at the left costal margin, and a tender liver edge was felt 3 cm. below the right costal margin. The genitalia were normal. There was slight ankle edema.

Laboratory studies revealed the following data: The volume of packed red cells was 54 ml. per 100 ml., and the white blood cell count was 14,500 per cu. mm. Differential count revealed 5 per cent juvenile forms, 74 per cent segmented neutrophils, 16 per cent lymphocytes, 5 per cent monocytes, and no eosinophils. Absolute eosinophil counts ranged from 3 to 10 per cu. mm. The urine contained 2+ albumin, a trace of sugar, 1 to 2 red blood cells per high power field and 4 to 5 white blood cells per high power field. Glycosuria was detected on only two occasions, and albuminuria was intermittent. Blood chemi-

TABLE I*

Laboratory Data		Normal	Case I (Carcinoma)	Case II (Adenoma)	Case III (Hyperplasia)
Fasting plasma 17-OH-corticosteroid (γ/100 ml.)		10–15	*36* (22–45)[17]	*29* (26–32)[2]	*26* (25–27)[4]
Urinary 17-OH-corticoids (mg./24 hr.)	Control	*5.3*†	*33.5* (10.5–53.5)[4]	24§	*30.1* (23.8–41.0)[8]
	After ACTH	*21.0*†	27	64.9**	104
Urinary total 17-ketosteroid (mg./24 hr.)	Control	*10.1*‡	*236* (203–254)[4]	9.3	*25.4* (17.5–34.2)[8]
	After ACTH	*15.4*‡	268	9.6	42

* Plasma and urinary corticosteroid data for three patients with Cushing's syndrome are contrasted with the findings in normal adults. Figures in parentheses represent the range of values obtained on different days; the number of days is indicated by the superscript. Average values are depicted in italics. All urinary 17-OH-CS were determined by the β-glucuronidase method.

† Sandberg et al.[12]

‡ Migeon, C. unpublished data.

§ 12 mg./24 hr. by the Reddy method.

** 33.7 mg./24 hr. by the Reddy method.

cal studies (sugar, urea, sodium, potassium, chloride, CO_2 combining power, serum proteins, calcium, phosphorus and uric acid) were within the normal range. The blood alkaline phosphatase was 12.5 Shinowara-Jones-Reinhart units. There was 10 per cent bromsulphalein retention at forty-five minutes (5 mg./kg.); terminally the bromsulphalein retention rose to 32 per cent. A standard oral glucose tolerance test revealed a fasting blood sugar of 78 mg. per cent (true glucose method); blood sugar values at one hour and at three hours were 235 and 245 mg. per cent, respectively. Hormonal studies are summarized in Figure 1 and Table I.

X-ray survey of the skeletal system revealed no osteoporosis. A chest film showed that both lungs were studded with multiple dense circular shadows, representing advanced metastatic disease. Excretory urography revealed inferior displacement of the right kidney by a large soft tissue mass which lay above that kidney. Skull films were normal.

The patient lost weight and gradually deteriorated over a two-month period. Progressive pulmonary insufficiency and bilateral pleuritic pain were the principal therapeutic problems. Emotional lability and transient episodes of psychotic behavior appeared. During the week before his death marked edema of the lower extremities and oliguria became evident. On March 22nd he became unresponsive and hypotensive. He died several hours later.

At autopsy the right adrenal gland was found to be replaced by a 540 gm. adenocarcinoma with multiple small areas of necrosis and hemorrhage. The inferior vena cava was completely obstructed by a tumor thrombus at the level of the liver. Metastases to the lung, liver, lymph nodes and left adrenal gland were found.

Adrenocortical Adenoma

CASE II. M. K., a forty-one year old white housewife, was seen for the first time on July 24, 1954. She had noticed swelling of the eyelids for about two years, followed by gradual swelling of the face and abdomen. There had been a 10 pound weight gain. Slight evening ankle edema became noticeable one and a half years before admission. During the year preceding admission amenorrhea, dyspnea on exertion, weakness, polydipsia, easy bruising, and facial and truncal hirsutism appeared.

Physical examination revealed an alert intelligent white woman. The blood pressure was 188/124, the pulse rate 88. There was disproportionate obesity of the trunk with a prominent cervicodorsal fat pad, a thick fat neck and a full, rounded, dusky face. The extremities appeared to be normal. The skin was warm and dry. A black stubble was seen

on the chin. There were numerous ecchymoses in the skin but no striations were seen. Examination of the ocular fundi revealed moderate arteriolar narrowing and tortuosity and marked arteriovenous compression. The heart and lungs were normal except for a snapping second heart sound at the aortic area.

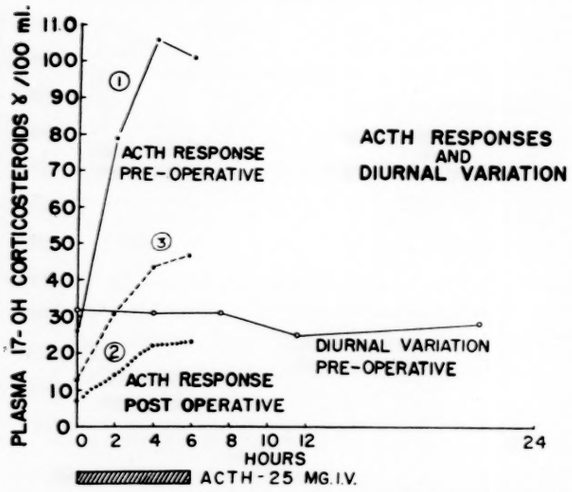

FIG. 2. Case II, M. K. (adrenocortical adenoma). Three curves illustrating the response of the plasma 17-OH-CS level to a standard infusion of ACTH are depicted. *Curve 1:* July 29, 1954—preoperative; *Curve 2:* August 25, 1954—fifteen days postoperative; *Curve 3:* September 13, 1954—thirty-three days postoperative (after ACTH therapy). The preoperative diurnal variation is also shown.

Laboratory and x-ray studies revealed the following data: Routine studies of the blood and urine were negative. Blood chemical values were normal except for a fasting blood sugar of 147 mg. per cent. X-ray examination of the skull and spine revealed slight osteoporosis. The sella turcica was normal. An excretory urogram was normal. Preoperative hormonal studies are summarized in Figure 2 and Table 1.

On August 10, 1954, after she had been prepared with cortisone and potassium chloride, the patient underwent surgery. A 10.5 gm. adenoma of the left adrenal gland was removed. The opposite adrenal was not explored. Postoperatively she received potassium chloride and gradually diminishing doses of cortisone. Cortisone was discontinued two days before the second ACTH test was performed on August 25, 1954, and then resumed in small doses (12.5 mg. every other day) until September 5, 1954. At this time, because of weakness, nausea and mild mental depression, HP acthar gel,® 25 to 40 mg. every other day, was administered with considerable clinical improvement. After three injections had been given the third ACTH test was performed on September 13, 1954. There had been a moderate improvement in her appearance, and her blood pressure had fallen to levels averaging 165/105.

Adrenocortical Hyperplasia

CASE III. N. J., a thirty-three year old white male laborer, was admitted on January 19, 1954. For six months he had noticed gradually increasing shortness of breath on exertion, orthopnea and a gain in weight of 47 pounds. Three months before admission purple streaks appeared over his lower abdomen. For one month he had noticed nocturia and for two weeks increasing hoarseness. He had noted no muscular weakness or change in potency. Five years before the present illness he had had a brief psychotic episode.

Physical examination revealed a short, markedly obese white man with disproportionate obesity of the face, neck and trunk. The blood pressure was 174/108, the pulse rate 120. His face was red, and a faint cyanosis of the lips and nail beds was present. The eyes were moderately proptotic. The face was "mooned" and a prominent cervicodorsal fat pad was seen. An acneform eruption was present over the upper trunk, and purplish striations were seen over the lower abdomen, thighs and upper arms. There was moderate pitting edema of the lower legs.

Laboratory studies revealed the following data: The volume of packed red cells was 47 ml. per 100 ml. blood and the white blood cell count 14,000 per cu. mm. Differential count revealed 1 per cent juvenile forms, 85 per cent segmented neutrophils, 14 per cent lymphocytes, and no eosinophils. Urinalysis was negative. Blood chemical studies (the same as those performed in Case 1) were normal. A standard glucose tolerance test was normal. An electrocardiogram showed left ventricular hypertrophy. Preoperative hormonal studies are summarized in Figure 3 and Table 1.

X-ray survey of the skeletal system did not reveal osteoporosis. Skull films and excretory urograms were normal. Retroperitoneal pneumography revealed a triangular shadow, 5 cm. in width and 3.5 cm. in height, overlying the left kidney. A smaller mass, less well defined, was seen overlying the right kidney.

After preoperative studies and preparation with a low sodium diet and intramuscular cortisone, the patient underwent left subtotal adrenalectomy with removal of 95 per cent of the gland on February 17, 1954. His postoperative course was smooth, and on March 5, 1954, right subtotal adrenalectomy was performed, again with removal of 95 per cent of the gland. Postoperatively cortisone was gradually reduced over a twenty-six-day period. Desquamation of the skin, weakness and mild diarrhea were present for a seven-day period after the cortisone therapy was discontinued.

By May, 1954, two months after the second operation, his appearance had changed considerably. His disproportionate obesity and "moon" facies had disappeared, and he had lost 26 pounds. The blood pressure had fallen to 126/84 and he was asymptomatic.

Three months postoperatively (June, 1954) he was readmitted to the hospital because of nausea, diarrhea and weakness of three days' duration. His blood pressure was 118/60, the pulse rate 110. There was diffuse pigmentation of the skin of the type seen in Addison's disease. Study of the serum electrolytes was normal except for mild hyponatremia (132 mEq./L.). After therapy with parenteral fluids, hydrocortisone administered intravenously and desoxycorticosterone, he improved rapidly and was asymptomatic within two days, by which time his blood pressure had risen to 140/80.

He has since been followed in the outpatient department. It has been possible to decrease his dose of oral hydrocortisone to 10 mg./day. Because of borderline hypertension and slight dependent edema, it has not been necessary to use maintenance desoxycorticosterone.

Pathologic findings were as follows: The excised portion of the left adrenal gland weighed 10 gm. No nodules were present. The average cortical thickness was 2.5 mm. Microscopic examination revealed normal cortical architecture. Under polarized light a polychrome-stained frozen section showed a few doubly refractile aggregates, particularly in the zona glomerulosa. The finding of a decrease in the cortical lipid and the brown color of the gland were considered to be consistent with cortisone effect, but there was no definite anatomic evidence of hyperplasia or neoplasm. The excised portion of the right adrenal gland weighed 10.5 gm. The cortex was thicker than normal with a fairly wide fasciculate zone in which the usual straight columnar pattern was partly obscured by a solid arrangement of sheets of cortical cells. Vacuolation was patchy. The nuclei were of uniform size. It was concluded that the appearance was consistent with, but by no means diagnostic of, adrenocortical hyperplasia with cortisone effect.

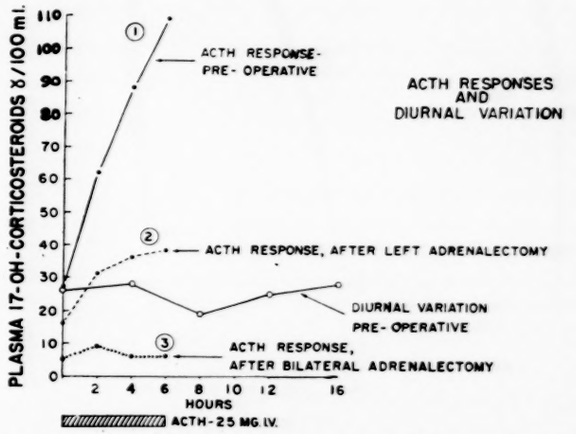

FIG. 3. Case III, N. J. (adrenocortical hyperplasia). Three curves illustrating the response of the plasma 17-OH-CS level to a standard infusion of ACTH are depicted. *Curve 1:* January 26, 1954—preoperative; *Curve 2:* March 3, 1954—after left adrenalectomy; *Curve 3:* April 16, 1954—after bilateral adrenalectomy. The preoperative diurnal variation is also shown.

RESULTS

1. Fasting plasma 17-OH-CS: In each of the three patients the fasting plasma levels of 17-OH-CS were significantly and persistently elevated, averaging two times the normal value. (Table I.)

2. Diurnal variation of plasma 17-OH-CS: In two patients, M. K. (Fig. 2) and N. J. (Fig. 3), the diurnal variation of plasma 17-OH-CS was studied. In normal subjects the highest plasma levels are observed in the early morning, decreasing steadily toward evening.[13–15] It is apparent that in these two patients with Cushing's syndrome this daily swing was not present. In the patient with carcinoma several isolated determinations at different times on different days also indicated a lack of diurnal variation.

3. ACTH stimulation: Pronounced differences from normal subjects in the response to standard ACTH infusions were found in these patients. In the normal person one observes at the end of the six-hour ACTH infusion a three- to fourfold increase in the plasma 17-OH-CS from an average fasting value of 10 to 15 γ/100

TABLE II*

Laboratory Data	Case II (Adenoma)		Case III (Hyperplasia)		
	Preoperative	Postoperative	Preoperative	After Left Adrenalectomy	After Bilateral Adrenalectomy
Fasting plasma 17-OH-corticosteroid (γ/100 ml.)	*29* (26–32)[2]	7	*26* (25–27)[4]	*26* (16–32)[4]	*5* (3–6)[3]
Urinary 17-OH-corticoid (mg./24 hr.)	12	4.7	*30.1* (23.8–41.0)[8]	31.7	*6.6* (6.4–7.1)[3]
Urinary 17-ketosteroid (mg./24 hr.)	9.3	. . .	*25.4* (17.5–34.2)[8]	21.8	*2.7* (2.4–3.1)[3]

* Pre- and postoperative plasma levels and urinary corticosteroid excretion in the two patients who underwent surgery. The symbols are the same as those in Table I. Urinary 17-OH-CS excretion in patient M. K. was determined by the Reddy method.

ml.[4] Curve 2 of Figure 3 is a good example of a normal response. In our patients, however, two distinct patterns of abnormality were noted: (a) In patient V. H. (carcinoma, Fig. 1), the plasma levels were unaffected. (b) In patient M. K. (adenoma, Fig. 2) and in N. J. (hyperplasia, Fig. 3), marked rises in plasma 17-OH-CS occurred at the sixth hour (400 per cent and 300 per cent of control values, respectively). It is of interest to note that although such high plasma levels were reached, the *percentage* increase after ACTH was not significantly different from that obtained in normal subjects.

4. Urinary 17-OH-CS: In all three patients the daily urinary 17-OH-CS excretion was four to six times the average excretion of normal subjects when the β-glucuronidase technic was employed. (Table I.) As with the plasma 17-OH-CS, distinct differences from the normal response to ACTH stimulation were observed. In V. H. (carcinoma) the administration of ACTH did not increase the excretion of 17-OH-CS. In the two patients with benign adrenocortical lesions, however, the level of urinary corticoid excretion was markedly elevated following ACTH.

5. Urinary 17-KS: The data relevant to the urinary excretion of 17-KS in these patients are presented in Table I. The average daily excretion of 17-KS of the patient with adrenocortical carcinoma was extremely high (236 mg.). In the patient with adenoma the values were normal for a female, while in patient N. J. (hyperplasia) the levels were moderately elevated. Of interest is the fact that in the two patients with neoplasm, ACTH stimulation did not alter the excretion of 17-KS whereas the patient with hyperplasia exhibited an almost twofold increase.

6. Serial changes in plasma and urinary steroids after therapy: It was possible to study patients M. K. and N. J. before and after surgery. The details are presented in Figures 2 and 3 and in Table II.

Postoperatively each of these patients showed evidence of partial adrenal insufficiency as judged by the response of the plasma 17-OH-CS to ACTH stimulation. The decrease in urinary 17-OH-CS to normal levels in patient M. K. and to temporarily subnormal levels in patient N. J. would similarly tend to indicate a reduction in total adrenocortical steroid output, which coincided with weight loss, partial disappearance of the characteristic physical findings, and, in the case of patient N. J., a fall in blood pressure to normal levels. This period of hypofunction in the case of patient M. K. was apparently reversed after a week of HP acthar gel therapy, as indicated by a normal ACTH test on September 13, 1954. Immediately after cortisone had been withdrawn transient abdominal pain, weakness, diarrhea and desquamation of the skin for one week developed in patient N. J. These symptoms have previously been described in patients undergoing subtotal adrenalectomy.[16] A fasting plasma 17-OH-CS level of 9 γ/100 ml. which

failed to rise after ACTH stimulation confirmed the clinical impression of partial adrenocortical insufficiency. Later, however, the degree of insufficiency became more profound, for some of the clinical features of Addison's disease developed three months after surgery (two months after cortisone therapy was discontinued). This prolonged period of adrenocortical hypofunction was accurately reflected in the blood and urinary steroid levels which were persistently reduced except for the urinary 17-OH-CS which remained within normal limits.

Late in the course of his disease there was a marked decrease in urinary steroid excretion in patient V. H. (carcinoma). During the last week of life, at a time when he was terminally ill and manifested edema and oliguria, the 17-OH-CS excretion was 4.9 mg./day and the 17-KS excretion 2.6 mg./day. This reduction in urinary steroid excretion probably reflected a diminished glomerular filtration rate.[17] Blood 17-OH-CS levels continued to be high until death.

COMMENTS

It is known that the prognosis of untreated Cushing's syndrome is poor. Fifty per cent of the patients in Plotz' series[17] died within the first five years of their disease. Consequently the aggressive surgical therapeutic approach recently advocated by Sprague[1] and Plotz[17] is particularly welcome.

Heretofore clinical methods of differentiating between the several types of adrenocortical lesions which may cause hyperfunction were for the most part indirect and unsatisfactory. It would appear from our data that perhaps such a differentiation can be made by means of a complete study of plasma and urinary corticosteroids. It is realized that only three patients have been studied in this manner and that generalizations can hardly be made from such a limited series but the striking differences in the pattern of hormonal alterations in these patients are considered worthy of presentation and comment.

In Case I the presence of metastatic malignant disease was quite apparent from inspection of the chest film. However, the high levels of urinary 17-KS excretion and the lack of response of the hyperfunctioning adrenocortical tissue to ACTH stimulation suggested a continuously high level of corticosteroid production by neoplastic tissue which was functionally autonomous and incapable of responding to factors which are known to stimulate the normal adrenal gland. Forbes has pointed out that extremely high excretion of 17-KS is likely to be due to adrenocortical carcinoma.[3]

In Cases II and III, on the other hand, benign adrenocortical lesions manifested themselves not only by unvarying increased production of corticosteroid hormones but also by persistence of the capacity of the adrenocortical tissue to respond to ACTH stimulation, albeit in an exaggerated manner. It is noteworthy that in patient M. K. (adenoma) ACTH stimulation effected a rise in the urinary 17-OH-CS but not in the 17-KS. This is in contrast to patient N. J. (hyperplasia) in whom the excretion of 17-OH-CS as well as 17-KS was increased with ACTH stimulation.

A somewhat more oblique approach to this problem of distinguishing between the various types of adrenocortical lesions in Cushing's syndrome has been employed by a number of investigators whose results have been reviewed recently by Jailer together with a report of his own findings.[2] Administration of cortisone was found to induce a significant fall in urinary 17-KS excretion in all patients with adrenal virilism secondary to adrenal hyperplasia and in most instances of Cushing's syndrome due to hyperplasia, while in patients with a functioning adrenocortical neoplasm (adenoma or carcinoma) a fall in 17-KS excretion was not observed in any instance. The administered cortisone was thought to depress the pituitary production of ACTH with resultant decrease in adrenocortical ketosteroid production. It was assumed that in the cases due to tumor there was a disruption of the normal pituitary-adrenal relationship. Unfortunately our data on 17-KS excretion during the administration of cortisone are too incomplete to be of value in confirming this finding. However, it is of interest that in our patients with tumors, both benign and malignant, the urinary 17-KS excretion was not influenced by ACTH stimulation.

CONCLUSIONS

1. The levels of plasma 17-hydroxycorticosteroids and the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids have been studied in three patients with Cushing's syndrome.

2. The patient with adrenocortical carcinoma exhibited an inflexible, autonomous type of in-

creased steroid production and excretion unaffected by ACTH.

3. The two patients with benign adrenocortical lesions (adenoma in one instance and hyperplasia in the other) exhibited a flexible type of increased corticosteroid production and excretion which responded in an exaggerated manner to ACTH stimulation. These patients underwent surgery with subsequent improvement.

4. It is suggested that studies of this type may be helpful in distinguishing cases of Cushing's syndrome due to malignant tumor from those due to benign lesions.

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Addison's Disease Associated with Histoplasmosis*

Report of Four Cases and Review of the Literature

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It is the purpose of this paper to report four cases of Addison's disease occurring in patients with histoplasmosis, and to stress the importance of considering this fungus disease as a possible etiologic agent in Addison's disease. Rawson et al.[1] called attention to the fact that histoplasmosis may be a cause of adrenal insufficiency. However, no cases of adrenal insufficiency associated with histoplasmosis have been reported in which the patients have been treated and survived. Three of the four patients to be reported herein are alive and in fair health five years, three years and three months, respectively, after the diagnosis of the two diseases had been established. One patient succumbed approximately twenty-two months after the diagnosis had been established.

CASE REPORTS

Case 1. W. S., a fifty-four year old white male, who lived in Virginia but had spent some time in Missouri and Panama, was first admitted to the University of Virginia Hospital on May 6, 1953, with a complaint of vomiting of six weeks' duration. He had noticed gradually increasing weakness and anorexia, and had sustained a thirty to thirty-five pound weight loss during the previous ten months. Six weeks prior to admission intermittent cramping abdominal pains developed and recurrent vomiting occurred.

Physical examination revealed an asthenic white male with a temperature of 98°F. and a blood pressure of 135 mm. Hg systolic and 70 diastolic. There were several small healing ulcerations on the buccal mucosa. The liver edge was palpable one fingerbreadth below the right costal margin. Moderate epigastric tenderness and guarding were present. No significant lymphadenopathy or pigmentary changes were noted.

Significant laboratory findings on admission included: hemoglobin 14 gm. per cent, hematocrit 46 per cent, white blood cells 9,800 with 35 per cent lymphocytes, 57 per cent neutrophils and 8 per cent eosinophils. The urine was acid with a specific gravity of 1.011; there was a trace of albumin, no sugar or acetone. A centrifuged specimen showed four to six white blood cells and two to four coarsely granular casts per high-powered field. Blood studies revealed a urea nitrogen of 80 mg. per cent, a fasting blood sugar of 80 mg. per cent, chlorides of 97 mEq./L., sodium of 134 mEq./L., potassium of 6.2 mEq./L. and a carbon dioxide combining power of 23.9 mEq./L.

An electrocardiogram showed low voltage complexes. The basal metabolic rate was minus 10 per cent. The Thorn test with intravenous ACTH was positive in that there was no fall in the eosinophil count. The Kepler-Power-Robinson water test was positive, the "A" factor being 6.2. Urinary 17-ketosteroids in two twenty-four-hour specimens were 7.0 mg. and 5.9 mg., respectively. Skin tests with O.T. 1:10,000 and histoplasmin were recorded as positive in twenty-four hours. X-rays of the chest, gastrointestinal series, barium enema and x-rays of the gallbladder were negative.

A diagnosis of Addison's disease was made and the patient was started on a regimen of oral cortisone, 12.5 mg. twice a day, and NaCl, 1 gm. three times a day. He followed a course of general improvement throughout hospitalization.

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He was seen again on August 17, 1953, with recurrence of symptoms. It was found at this time that he required desoxycorticosterone acetate as well as cortisone and added salt for adequate control of symptoms. He was therefore given 30 mg. of corticosterone-trimethyl-acetate intravenously, and oral cortisone, 12.5 mg. twice a day. He continued to improve on this regimen and remained well until June 28, 1954, when he was admitted to the hospital with a complaint of painful ulcers on his lip and tongue. Examination of the mouth lesions revealed a 1 by 1 cm. indurated ulcer, ½ cm. in depth, on the vermilion border of the upper lip, and a similar larger lesion on the left lateral aspect of the tongue. Biopsy of these lesions revealed organisms which morphologically were Histoplasma capsulatum. Cultures of the mouth lesions and the urine revealed this organism. A histoplasmin skin test was equivocal in forty-eight hours. X-rays of the chest were unchanged from the previous admission.

At the suggestion of Dr. E. C. Cawley of the Department of Dermatology the patient was given stilbamidine in daily doses of 50 mg. The dose was gradually increased to 150 mg. daily but symptoms of toxicity developed after a total dose of 825 mg. Then dihydroxystilbamidine* was administered in a daily dose of 50 mg. The dose was increased gradually to 150 mg. daily for a total dose of 2,025 mg. There were no obvious signs of toxicity while he was receiving dihydroxystilbamidine. The lesions in the mouth showed evidence of healing and became much less tender. On a follow-up visit one month after discharge from the hospital, the ulcers in the mouth had completely healed and there was no new evidence of histoplasmosis. Addison's disease remained well controlled on a program of oral cortisone and intramuscular desoxycorticosterone-trimethyl-acetate.

The patient remained in fair health until January, 1955, when he had a recurrence of sore throat, hoarseness and inability to swallow solids. He was readmitted to the University of Virginia Hospital and examination revealed ulceration about the false vocal cords. Biopsy of these lesions again showed H. capsulatum. The patient was again treated with hydroxystilbamidine® with slight initial improvement but the lesions then gradually increased in size to involve the larynx and pharynx. It was necessary to insert a Levin tube in order to administer a liquid diet.

The patient was transferred to the Clinical Center at the National Institute of Health. He died in acute renal failure three weeks after admission. Autopsy* was obtained and the findings pertinent to this case history are as follows: (1) Sections of adrenal gland show almost complete destruction of adrenal tissue by extensive caseation surrounded by chronic granulomatous inflammation, with a few epithelioid tubercle-like lesions and occasional giant cells. The caseous areas contain cellular debris and tiny fragments, some of which contain organisms resembling H. capsulatum. (2) Sections of the kidney show areas of chronic granulomatous inflammatory reaction, with caseous and non-caseous focal necrotic lesions containing a number of organisms resembling H. capsulatum.

CASE II.† J. T. S., a forty-three year old white male resident of Mississippi, was admitted to the Foundation Hospital in New Orleans in August, 1947, with a complaint of hoarseness of five months' duration. Examination of the larynx by Dr. Francis LeJeune revealed a smooth infiltrating lesion of the left vocal cord. Sections of the lesion contained H. capsulatum. Treatment with promin,® 15 mg. daily, was followed by progressive improvement in the appearance of the vocal cord and gradual disappearance of the hoarseness. He remained well until March, 1948, when constipation, epigastric burning and morning weakness developed. One month later diarrhea, nausea and vomiting, and abdominal pain developed. He was readmitted to the hospital. Examination revealed a blood pressure of 80 mm. Hg systolic and 50 diastolic, and the typical pigmentary changes of Addison's disease. Blood studies revealed a fasting blood sugar of 72 mg. per cent, chloride of 85 mEq./L. and sodium of 104 mEq./L. X-ray of the chest showed a small

* We wish to thank Dr. W. G. Morson of William S. Merrell Company for supplying the dihydroxystilbamidine.

* Sections of tissue and case history kindly furnished by Dr. Horace Bernton and Dr. John Edgecomb of the National Institute of Health, Bethesda, Maryland.

† This case is reported by the kindness of Dr. Albert Segaloff, Ochsner Clinic, New Orleans, La. The laryngeal lesion has been discussed by Dr. Francis LeJeune of the Ochsner Clinic, New Orleans, La. in a previous paper, "Histoplasmosis—A Deficiency Disease," by Roberts, A. E. and Forman, F. S. *Ann. Otol., Rhinol., & Laryng.*, 59: 809, 1950.

heart with clear lung fields. A Kepler-Power-Wilder test was positive; examination of the larynx was completely negative. The diagnosis of Addison's disease was made and the patient was regulated on a regimen of DOCA and NaCl. He did well on this regimen until February, 1950, when he was seen in the clinic on account of falling blood pressure for the past few months and fatigue in the morning. His blood pressure was 85 systolic and 55 diastolic; temperature was 103.7°F. At this time the patient was thought to be on the verge of an addisonian crisis and was treated with aqueous and lipoadrenal extract. His disease was finally regulated with desoxycorticosterone acetate, 2 mg. intramuscularly, oral cortisone, 25 mg., and NaCl, 2 gm., daily. The patient has continued to do well on this schedule with good control of Addison's disease and no evidence of histoplasmosis when last seen in February, 1954.

CASE III. A. C., a forty-four year old white male high school instruction director who lived in Virginia but had been stationed in Memphis, Tennessee, during World War II, was admitted to the University of Virginia Hospital on July 11, 1951, complaining of a sore throat of two months' duration. The patient was apparently in good health until November, 1946, when he had the onset of cough and fever and was thought to have had "virus pneumonia." He continued to have residual pneumonitis by x-ray examination following his recovery from this illness. After repeated examinations, including biopsy of a posterior cervical lesion, a diagnosis of sarcoid was made. Treatment during this period was mainly supportive. The patient made quite a rapid improvement and remained in good health until May, 1951, when another episode of "virus pneumonia" occurred. After recovery from the acute phase of this episode dysphagia developed which failed to respond to local therapy. Increasing weakness, a twenty-five pound weight loss and a nocturnal fever of 100°F. were noted. In June, 1951, a lesion which had appeared on the epiglottis was biopsied and reported as histoplasmosis. The patient was referred to the University of Virginia Hospital for further treatment.

Physical examination on admission revealed an obviously malnourished, debilitated, middle aged white man in no acute distress. Blood pressure was 110 mm. Hg systolic and 70 diastolic. There were two small grayish brown excoriated areas with thick scabs on the parieto-occipital area of the scalp and two similar lesions on the face in the region of the alae. No significant lymphadenopathy was noted. Indirect laryngoscopy revealed the epiglottis to be moderately thickened with a pink, irregular, granulomatous lesion on the superior rim and extending about ½ cm. down the posterior surface.

Significant laboratory findings included: hemoglobin 15 gm. per cent, red blood cells 4.56 million, white blood cells 7,300, blood urea 55 mg. per cent, chloride 81 mEq., and CO_2 combining power 23 mEq./L. The urine was acid, with a specific gravity of 1.020. Microscopic examination revealed two white blood cells, no red blood cells, numerous hyaline and a few granular casts. The histoplasmin skin test was negative in a dilution of 1:10,000 although the complement fixation test for histoplasmosis was positive in a dilution of 1:2048. X-ray of the chest revealed a slight old pleural reaction in the right base. A biopsy of one of the posterior scalp lesions was interpreted as histoplasmosis of the skin.

Treatment with a 40 per cent solution of ethyl vanillate was started on July 25, 1951. On August 1st the patient went suddenly into acute Addison crisis, manifested by a shock-like state, unobtainable blood pressure, a serum chloride of 81 mEq./L., and a CO_2 combining power of 14 mEq./L. Treatment with adrenal cortical extract, whole blood, and glucose in physiologic saline produced a remission and within twenty-four hours it was possible to maintain the patient on a regimen of DOCA and cortisone. Subsequently, the DOCA was discontinued and the symptoms were well controlled on 20 mg. of cortisone daily.

The patient was transferred to the Veterans Administration Hospital, Roanoke, Virginia, on August 13, 1951. Recurrence of symptoms of addisonian crisis occurred on August 25th and again on September 16th, responding each time to appropriate therapy. Continued treatment of the histoplasmosis with ethyl vanillate gave generally satisfactory results and gradual healing took place in all the cutaneous lesions. The lesion of the epiglottis failed to respond to this therapy. This lesion was treated with propamidine and varidase® sprays and a marked diminution in the size of the epiglottic granuloma occurred. On discharge from the hospital on March 6, 1952,

the patient showed no visible evidence of histoplasmosis.*

He has been followed at six-month intervals and his condition has remained unchanged. Addison's disease is well controlled by two linguets of DOCA and 25 mg. of oral cortisone daily.

CASE IV. D. H. G., a forty-five year old white farmer, was admitted to the local Tuberculosis Sanatorium on March 4, 1955. Four months prior to admission listlessness, weakness and malaise developed and the patient noted a low-grade afternoon fever with sweating but no chills. A severe non-productive cough developed and a fifteen pound weight loss was sustained during the two months prior to hospitalization. There had been no preceding febrile illness or cough.

The patient had spent most of his life in the farming area of Virginia except for a short visit to Nashville, Tennessee, in 1949.

Physical examination on admission revealed a blood pressure of 130/90. A few scattered rales were present at the apex of the left lung anteriorly and over the left mid-lung posteriorly. A grade III systolic murmur was noted along the left sternal border and auricular fibrillation was present with a pulse rate of 100. Single small left axillary and right inguinal nodes and a small subcutaneous epigastric nodule were palpable. The liver and spleen were palpable, smooth and non-tender. Bilateral inguinal hernias were present.

Laboratory data revealed normal blood counts and urinalysis, negative spinal fluid, and smears of concentrated sputum specimens were negative for acid-fast bacilli. Tuberculin skin tests and blood agglutinations for typhoid, paratyphoid, brucellosis, and tularemia were negative. X-ray of the chest showed miliary calcifications consistent with healed histoplasmosis.

On March 21, 1955, the patient was transferred to the University of Virginia Hospital. Physical examination at this time was essentially unchanged except that vitiligo interspersed with moderate pigmentation was noted on the wrists, elbows, lower abdomen, scrotum and dorsum of the penis.

Admission laboratory findings included hematocrit 52 per cent, white blood cells 6,200, with sixty-four per cent neutrophils, 31 per cent lymphocytes, 4 per cent monocytes and 1 per cent eosinophils; fasting blood sugar 110 mg. per cent; blood urea 37 mg. per cent; total serum protein 7.1 gm. per cent with albumin 4.1 and globulin 3.0; cephalin flocculation 4 plus, thymol turbidity 19.4 units and 14 per cent BSP retention in forty-five minutes. The erythrocyte sedimentation rate was 39 mm. in one hour (Bray).

Skin tests for histoplasmosis, coccidioidomycosis and blastomycosis were negative. Agglutinations for histoplasmosis and coccidioidomycosis were negative. Nine blood cultures showed no growth.

A bone marrow biopsy revealed slight normoblastic hyperplasia. Biopsy of the inguinal node showed granulomatous changes compatible with sarcoidosis.

On April 15, 1955, exploratory laparotomy was performed to obtain biopsies of the liver and abdominal lymph nodes. These showed granulomatous lesions containing giant cells but no evidence of necrosis and special staining technics failed to reveal fungi or acid-fast organisms.

The patient's postoperative course was uneventful until eighteen hours following surgery when acute adrenal insufficiency suddenly developed as evidenced by confusion, lethargy, temperature 104°F., blood pressure 60/40, serum sodium 110 mEq./L., serum potassium 4.87 mEq./L. and blood urea 62 mg. per cent. Intravenous hydrocortisone with 5 per cent glucose in physiologic saline produced a prompt response with clearing of the sensorium, rise in blood pressure and a fall in temperature to normal levels. The blood pressure was stabilized and normal serum electrolyte values were maintained on 37.5 mg. of cortisone daily by mouth. In retrospect, this man had adrenal insufficiency before he was operated upon as the serum sodium was 120 mEq./L., serum potassium 6.5 mEq./L., blood urea nitrogen 57 mg. per cent, and serum chlorides 89 mEq./L. It was not recognized until the stress of surgery precipitated acute adrenal insufficiency.

DISCUSSION

In recent years due to the decreased incidence of tuberculosis and the advances made in the early diagnosis and adequate treatment of this disease, there has been reduction in the number

* Dr. Robert Scott kindly supplied this portion of the history, and has previously reported some aspects of this case. "Treatment of Progressive Histoplasmosis with Ethyl Vanillate and Propamidine," by Ellis, F. F., Scott, R. J. and Miller, J. M. *Antibiotics & Chemother.*, 2: 347, 1952.

TABLE 1

Author	Total No. of Cases of Histoplasmosis	Autopsies	Cases with Adrenal Involvement at Autopsy	Cases with Clinical Symptoms Suggestive of Adrenal Insufficiency*
1. Parsons, Zarafonetis[5]	71	56	18	2
2. Manchester[11]	1	1	1	1
3. Palmer et al.[12]	1	1	1	1
4. Van Pernis et al.[13]	1	1	1	1
5. Thomas, Morehead[14]	1	1	1	1
6. Martin, Sieber[15]	1	1	1	1
7. Ziegler[16]	1	1	1	1
8. Rawson et al.[1]	1	1	1	1
9. O'Donnell[3]	1	1	1	1
10. Vivian et al.[4]	20	Not stated	6	2
11. Pinkerton, Iverson[19]	3	3	3	2
12. Clinco-pathologic Conference[21]	1	1	1	1

* In these cases the diagnosis of adrenal insufficiency was not proved by laboratory tests of adrenal function.

of cases of adrenal insufficiency due to bilateral tuberculous necrosis of the adrenals. In early reports tuberculosis is given as the cause in 70 to 90 per cent of the cases of Addison's disease.[2] Tuberculosis is now reported to be responsible for about 50 per cent of the cases.[3] With a reduction in the number of cases of Addison's disease due to tuberculosis, the systemic fungus infections should be considered more carefully as an etiologic agent in all cases of adrenal insufficiency.

In this country the vast majority of cases of histoplasmosis occur in the Central Mississippi Valley.[6] In the cases reported herein, all of the patients had been in this area sometime prior to the onset of their illness. Three of the patients (A. C., W. S. and D. G.) had lived most of their lives in Virginia but had been in the Mississippi Valley area at some time, while the other patient (J. T. S.) lived in Mississippi. A history of having been in the Mississippi Valley area in any person with Addison's disease should raise the suspicion of histoplasmosis as the etiologic agent. Also of interest is a recent report stating that H. capsulatum was isolated from twenty-two of fifty cats and from twenty-two of fifty dogs obtained from Loudoun County, Virginia.[17] One patient (D. G.) lives in this county and W. S. was from an adjoining county.

Although at autopsy the adrenal glands are among the most frequently involved organs in cases of histoplasmosis, case reports of Addison's disease due to this organism, which have been recognized ante mortem, are relatively rare. (Table 1.) Rawson et al.[1] reported one case and were able to find reports of eight other cases published prior to 1948. O'Donnell reported a case with massive caseous necrosis of both adrenals due to histoplasmosis, which was diagnosed as Addison's disease after necropsy.[3] In a review of all the cases of histoplasmosis at the Mayo Clinic, Vivian et al. reported adrenal involvement in six of twenty cases.[4] There was clinical evidence of Addison's disease in two of these cases. Parsons and Zarafonetis reported three cases of patients with extensive caseous necrosis of the adrenals and, in the cases reviewed, found adrenal involvement in eighteen of seventy-one.[5]

The diagnosis of histoplasmosis is established by isolation of the organism by culture or demonstration of the organism in infected tissue. Histoplasmin skin tests and complement fixation tests may be useful aids in diagnosis in some cases but the results with these tests are variable and are frequently difficult to interpret due to cross reactions. Campbell and Binkley, in a study of thirty-seven cases proved by culture, reported four different antibody patterns in histoplasmosis.[7] In ten cases of generalized histoplasmosis associated with severe endocrinopathy there were consistently low complement fixation titers, and titers were also negligible in the sera of patients showing only cutaneous or mucocutaneous lesions.

In our cases the diagnosis of Addison's disease

was made prior to the diagnosis of histoplasmosis in one instance, and after the diagnosis of histoplasmosis in two. It should be noted from Case III that addisonian crisis occurred suddenly without any previous overt signs of adrenal insufficiency. This danger should be recognized and watched for in any patient with histoplasmosis. It is also to be noted that two of our patients (A. C. and D. G.) were diagnosed as having had sarcoidosis before the diagnosis of histoplasmosis was established. The possible confusion of histoplasmosis with sarcoidosis has been previously emphasized.[18–20]

It will be noted that each patient received a different form of treatment for the systemic histoplasmosis, including ethyl vanillate, hydroxystilbamidine and promin. The drug therapy may have had very little to do with the course of the disease since the three very different drugs all seemed to be effective initially. Patient W. S. had what appeared to be a clinical response to hydroxystilbamidine, but recurrent pharyngeal lesions did not respond to a second course of this therapy. It may be proper to try hydroxystilbamidine until a specific treatment for systemic mycoses is available. The treatment of hypoadrenalism in these cases does not differ from the treatment of Addison's disease due to other causes. As would be expected, there has been no amelioration of Addison's disease associated with apparent quiescence of the histoplasmosis.

The incidence of adrenal involvement at autopsy is not as frequent in other systemic mycoses as in histoplasmosis. Martin and Smith[8] reported adrenal involvement in two of sixty proved cases of blastomycosis. Kunkel et al.[9] also found only two cases of adrenal involvement in ninety cases of blastomycosis seen at the Mayo Clinic. Cryptococcosis may involve the adrenals, and Rawson and co-workers[1] were able to find a total of six cases. Coccidioidomycosis with adrenal involvement occurred sixteen times in ninety-five cases reported by Forbus and Besterbreurtje.[10]

SUMMARY

1. Four cases of Addison's disease associated with proved histoplasmosis are reported. Three of the patients are alive and in fair health five years, three years and three months, respectively, after the diagnosis of the two diseases had been established. The autopsy of the fourth case revealed destruction of the adrenal glands by caseation necrosis. The necrotic material contained H. capsulatum.

2. Two of the four cases reported were first diagnosed as sarcoidosis on the basis of lymph node biopsy.

3. Histoplasmosis should be considered as an etiologic agent in Addison's disease as suggested by these cases and by a review of 103 cases of histoplasmosis in which 35 per cent of the cases were found to have adrenal involvement. This is a higher incidence of adrenal involvement than in the other systemic mycoses.

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Alterations in Thyroid I-131 Uptake, Basal Metabolic Rate and Serum Cholesterol Following Treatment of Hyperthyroidism with Radioactive Iodine*

Value in Early Prediction of Success or Failure of Therapy

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RADIOACTIVE iodine is an effective agent for the treatment of hyperthyroidism and now has an established place in the therapy of this disease. Response to therapy is judged largely by the regression in clinical symptoms and signs. However, these often regress gradually and evaluation of the eventual outcome consequently is delayed. Patients may remain in a hyperthyroid state for several months before it becomes apparent that a remission is not going to occur and further therapy is necessary. A reliable means of predicting failure of therapy within the first six to nine weeks after treatment or sooner would be of great practical value.

Few data have been published which enable us to evaluate the commonly available laboratory procedures in this respect. A general impression exists that tracer studies do not reflect accurately thyroid function following I-131 therapy. Yet what evidence has appeared favors the opposite view. It is clearly desirable that we know the changes occurring, after therapy, in the laboratory measures generally available and commonly utilized for diagnosis. The present study attempts to evaluate such changes. Specifically, post-therapy alterations of the thyroid uptake, basal metabolic rate and total serum cholesterol, as well as of the clinical state, have been studied and their value in predicting the ultimate result of a given therapeutic dose has been defined.

METHODS AND MATERIALS

Cases and Follow-up Procedure. Sixty-six hyperthyroid patients were studied. Only four were women. The diagnosis of hyperthyroidism was established before therapy in each patient on the basis of the clinical findings, thyroid uptake, basal metabolic rate, and in some instances, the serum protein-bound iodine as chemically determined, as well as various special radioiodine studies such as the conversion ratio, thyroid clearance and rate of uptake of I-131 by the gland. Sixty patients had diffuse toxic goiters and six had nodular toxic goiters. The few patients with nodular goiter were grouped with those with diffuse goiter because the test alterations following therapy were in no essential way different from those of the larger group.

The sixty-six patients received a total of eighty-nine therapy doses. The variables being evaluated were analyzed according to dose rather than patient since with each measure variability from dose to dose for a given patient was as great as variability from patient to patient. This is seen readily upon inspection of the raw data in Tables I, II and III. In fifty-one patients data utilized in the analysis were obtained following a first dose and in fifteen patients following a second or subsequent dose. All patients were treated similarly, receiving sufficient I-131 so that at twenty-four hours approximately 100 μc per gram was in the thyroid.

The patients were re-evaluated following therapy generally at six to nine week intervals and at each of these visits, whenever possible, determinations of thyroid uptake, basal metabolic rate serum cholesterol and, in more recent patients, protein-bound iodine (chemically determined) were obtained.

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After an adequate period of observation following each therapeutic dose the patients were classified into one of three groups based upon the clinical findings: those in remission, those with persistent hyperthyroidism, and those exhibiting myxedema. The term myxedema is used synonymously with significant hypothyroidism. The thyroid function tests were then analyzed in the light of this classification. The patients comprising the satisfactory remission and the myxedema groups were followed from twenty to 157 weeks. With four exceptions the patients of the remission group were followed at least thirty-six weeks. The exceptions were followed twenty, twenty-one, twenty-six and thirty-five weeks and were definitely euthyroid clinically. In our experience to date a relapse of hyperthyroidism has not occurred when a patient has become euthyroid unequivocally by clinical criteria. The average period of observation for the remission group was seventy-seven weeks.

Patients were placed in the myxedema group if myxedema was clinically evident five or more months following therapy. The group was followed an average of seventy-nine weeks (range twenty-one to 145 weeks). During most of this period the patients were receiving thyroid extract and from time to time attempts were made to discontinue the therapy. The average interval between radioiodine therapy and the final laboratory observations was thirty-six weeks as recorded in Table III (range twelve to seventy-one weeks).

Retreatment with radioiodine was withheld in the failure cases until it became apparent that a remission would very probably not ensue. The average interval of follow-up before retreatment was twenty-one weeks (range nine to fifty-five weeks).

Variables. The primary measurements to be evaluated are the thyroid I-131 uptake (TU), basal metabolic rate (BMR), total serum cholesterol (TC) and clinical status (CS). Though fragmentary, data for the chemical determination of protein-bound iodine (PBI) are included for comparative purposes but no attempt is made to apply the results to prediction in the individual case. Thyroid uptake measurements were by a method described elsewhere.[1] The basal metabolic rate was determined in the standard way with a Sanborn Metabolator. Serum cholesterol determinations were by the method of Abell and her coworkers.[2] An estimate of the clinical severity of the hyperthyroidism was based upon the degree of hyperactivity and heat intolerance, weight loss, tachycardia, increased appetite and weakness. Chemical determinations of protein-bound iodine were made by the incineration method of Barker, Humphrey and Soley.[3]

Since a decision based upon all of these variables was found to be more valuable than that based upon any one variable, a weighted combination of the measures, the protein-bound iodine excepted, was calculated at each time interval during the follow-up period. A six-fold rating score for each variable was derived from the following:

Ratings	CS	TU (%)	BMR (%)	TC (mg. %)
1	Ho, severe	0–6	< −29	400+
2	Ho, mild or moderate	7–10	−20 to −29	280–400
3	Eu	11–20	−1 to −19	200–279
4	Eu	21–49	0 to +19	140–199
5	Hr, mild or moderate	50–69	+20 to +39	120–139
6	Hr, severe	70+	+40+	<120

Ho = hypothyroid; Eu = euthyroid; Hr = hyperthyroid; CS = clinical status; TU = thyroid I-131 uptake; TC = total serum cholesterol.

The rating scores were combined, giving equal weight (of 1) to the clinical status, thyroid uptake and basal metabolic rate, and approximately one-third that weight to the cholesterol. The combined score was thus calculated by adding 0.3 of the rating for the serum cholesterol to the sum of the ratings for the clinical status, thyroid uptake and basal metabolic rate. For example, a patient who was moderately hyperthyroid (rating 5) with an uptake of 72 per cent in twenty-four hours (rating 6), basal metabolic rate of +19 per cent (rating 4), and serum cholesterol of 128 mg. per cent (rating 5) would have a combination score of $5 + 6 + 4 + 0.3(5) = 16.5$. This combined score was analyzed like the separate variables.

For purposes of the present report values falling within the following limits were taken as normal: thyroid uptake 10 to 50 per cent, basal metabolic rate −20 to +20 per cent, serum cholesterol 140 to 300 mg. per cent, protein-bound iodine 3.5–7.9 μg. per cent and combined score 9.5 to 13.5 units.

RESULTS AND INTERPRETATIONS

The raw data for each variable before and after each therapeutic dose are shown in Tables I, II and III. The data have been grouped according to the final clinical result of each therapeutic regimen, that is, satisfactory remission of hyperthyroidism, persistence of hyperthyroidism or progression to myxedema. These groups will hereinafter be referred to as the remission, failure and myxedema groups, respectively. It will be noted in Table I (remission group) that of the patients studied six to nine weeks following therapy 30 per cent were still clinically hyperthyroid and 23 per cent still had basal metabolic rates in the hyperthyroid range, but in only 8 per cent (patients 36 and 37) were the results of uptake studies within the hyperthyroid range. The basal metabolic rate of patient 36 had

Table I

CLINICAL STATUS AND THYROID FUNCTION TEST RESULTS BEFORE AND SERIALLY AFTER THERAPEUTIC DOSES OF RADIOIODINE THAT RESULTED IN REMISSION OF HYPERTHYROIDISM. REMISSION GROUP.

Dose			Before Therapy					After Therapy																											
								6-9 Weeks					10-13 Weeks					14-23 Weeks					24-35 Weeks					36 Weeks or more					Weeks	
Pt.	No.	Age	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	Followed	
		yrs		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		
1	I	30	H	88	+35	159		H	7	+43	172		E	24	+10	250																			
2	I	53	H	71	+39	120		H	24	−3	198							E	13	−4	197		E	30		173		E						50	
3	I	27	SH	73	+38	155		E	22	−22	234							E	48	0	210		E	54	−14	242		E	25		197			80	
4	I	34	H	87	+19	199		E	15	−16	135							E	24	−13	200		E	24	−3	186		E	46		254			110	
5	I	36	SH	79	+14	177		E	8	0	240							E		+4			E					E			220			113	
6	I	28	SH	58	+42	150		E	9	−10	255							E	23	−12	179							E	20	+7	209			125	
7	I	57	H	63	+15	130		H	32				H	37	+11	236												E	29	0	215			97	
8	I	33	H	73	+30	164		E	11	−13	270												E	38	+1	197		E		+17	221			141	
9	I	39	H	80	+38	134	15.5	E	1	+8	180							E	31		306		E	30	−5	214		E			192			79	
10	I	37	H	62	+39	114		H	31	+13	192							E	37	+19	197		E					E	42	+6	195	7.7		60	
11	I	34	SH	97	+41	122		E	6	−12	354							E	10	−8	309							E						54	
12	I	37	SH	83	+46	187		E	22	−11	326							E	30	−23	400		E	37	−7	283		E	34	−22	320	4.6		102	
13*	I	58	H	50	+9	280		E	18	0	172							E	35	−4	185							E	47	−9	243	5.3		94	
14*	I	56	H	66	+22	134		E	1	−5	174							E	30	+9	197							E						84	
15*	I	59	H	66	+25	129	9.2											E	31	−5	196							E	4	+6	173			99	
16	I	26	SH	63	+24	137		H		+21			E	2	+20	217												E	13	−21	242	2.8		80	
17	I	35	H	65	+31	147	14.2						E	32	+30	158		E		+7	192							E		0	206			60	
18	I	56	H	78	+29	294		E		+16	354		E	15	−12	259							E	24	+7	221		E	21	+21	235	3.6		66	
19	I	28	H	60	+36	139							E	4	−2	470							E	34	+6	382		E	30		374			93	
20	I	22	H	87	+32	184		E		+42			E	1	+10	366												E						42	
21	I	24	H	69	+37	162		E		+25								E	27	−14								E	37		205			90	
22	I	67	H	77	+53	158	12.2											E	12	−8	319							E	25	−4	251			101	
23*	I	32	H	61	+25	167												E	47	−5			E	25	+2	249		E	22	+18	200	6.3		47	
24	I	30	H	73	+32	154							E		+21			E	25	+4								E	23	0	284	3.4		157	
25*	I	56	H	50	+6	254												E	47	+3	235							E	51	+4				44	
26	I	42	SH	80	+47	175		E	15	+19	198							H	35	+29	326							E	33	+1	322	7.5		40	
27	I	30	SH	91	+56	143												E	65	+13	189							E	48	−14	224			55	
28	I	22	SH	71	+49	133																	E	42	−15	192		E		−4	200			42	
29	I	30	SH	83	+55	100		E		+7								E		+9			E	32	−7			E	26				3.9	125	
30	I	24	SH	75	+42	120	13.2	H	5	+15	170		E		−9			E		+1			E		+3			E	26	−18	192			115	
31	I	41	H	73	+42	230	20.7	E	6	−18	410	2.0											E	32	−8	225	2.0						35	
32	I	39	H	72	+20	195	18.5	H	12	−5	255	5.0						E	36	+4	243	6.0												21	
33	II	30	H	53	+36	178		E	16	−6	227							E	33	+4	218	6.2	E	31	−9	217	7.6							26	
34	II	45	H	66	+42			E	5	−6			E		−11	249							E	20	−4	205		E	23	+8	200	5.9		60	
35	II	38	H	69	+24	216		E	12	+2								E	51	+10	236							E	37		182			99	
36	II	26	SH	77	+51		14.2	H	68	+12	185	7.4	E	55	0	251	5.1	E	48	−3	199	7.9	E	37		220		E	31	+11	292			126	
37*	II	38	H	62	+29	130		H	52	+28	172							H	58		175		E	40	+18	184	7.8	E	50	+6	198	6.9		45	
38	II	62	H	66	+27	168	9.5											E	18	−11	174		E	41	0	167		E	17	+9	195			66	
39	III	46	H	67	+74	170	10.5	E	15	+30	245	4.5	E	26	−2	167	5.6																	20	
40	III	29	H	65	+17	135												H	57	+4	130	5.9	H	53	+28	144	4.3	E	43	+31	225	5.6		38	
41	IV	30	H	54	+33	202		E		+15								E	24									E	40	+6	158	4.2		48	
49	II	59	H	60	+11	204	10.0	E	29	−7	270	6.1						E	45	+13	185	6.8						E	30	−2	172			105	
60	I	33	H	65	+33	208		E			334		M	17	−20	455		E			367							E	47		193	7.1		38	
61	I	32	SH	85	+38	143		H		+40	177		E	2	−9	282		M	27	+1	366		E	35	+6	267	6.0	E	20	+9	280	7.0		96	
																							E	13		361		E		−3	260			128	
N		44		44	44	42	11		25	31	25	5		11	14	12	2		28	29	26	5		20	18	19	5		30	27	34	15		44	
Mean		38		71	+34	167	13.4		18	+7	236	5.0		20	+3	280	5.4		35	+1	236	6.6		34	0	228	5.5		31	+2	227	5.4		77	

Abbreviations are as follows: Pt., patient. CS, clinical status. TU, thyroid uptake. BMR, basal metabolic rate. TC, total cholesterol. PBI, protein-bound iodine. H, hyperthyroid. SH, severely hyperthyroid. E, euthyroid. M, myxedematous. SM, severely myxedematous.
* Nodular goiter.

Table II

CLINICAL STATUS AND THYROID FUNCTION TEST RESULTS BEFORE AND SERIALLY AFTER THERAPEUTIC DOSES OF RADIOIODINE THAT DID NOT RESULT IN REMISSION OF HYPERTHYROIDISM. FAILURE GROUP.

			Before Therapy					After Therapy																			
								6-9 Weeks					10-13 Weeks					14-23 Weeks					24 Weeks or more					Weeks
Pt.	Dose No.	Age	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	Followed
		yrs		%	%	mg%	µg%		%	%	mg%	µg%		%	%	mg%	µg%		%	%	mg%	µg%		%	%	mg%	µg%	
33	I	30	SH	73	+64	135	25.7	H	59		209							H	53	+36	178							21
34	I	44	SH	78	+24	169												H	75	+22			H	66	+42			24
35	I	37	SH	69	+46	277		H	9	+43													H	69	+24	216		30
36	I	26	SH	77	+58	184	18.2	SH	77	+51		14.2																9
37*	I	38	SH	73	+42	140							H	62	+29	130												13
38	I	62	H	87	+29	151	14.7	H	9	+9	179							H	66	+27	168	9.5						21
39	I	46	SH	81	+48	129	17.1	H	69	+42	158		H	82														13
39	II	46	H	82														H	67	+74	170	10.5						15
40	I	28	SH	68	+48	83		H	78	+60	113							H	81	+57	127		H	71	+43	137		30
40	II	29	H	71	+43	137		H	64	+17	122	8.7						H	65	+17	135							21
41	I	29	SH	74	+57	178		SH		+73	125							SH	68	+60	140							14
41	II	29	SH	68	+60	140		SH	54	+67	134																	6
41	III	29	SH	54	+67	134												H	37	+39	196		H	54	+33	202		36
42	I	20	SH	69	+58	205		SH	82	+41	129							SH	85		130							23
42	II	21	SH	85		130							SH	82	+63	137												12
42	III	21	SH	82	+63	137		SH	65	+48	151																	8
42	IV	21	SH	65	+48	151							H	46	+18	174		H	51	+19	145		H	56	+25	145	9.5	48
43	I	26	SH	82	+46	176	13.5	SH	74	+38	124							SH	65	+39	122							15
43	II	26	SH	65	+39	122		H	59	−12	176	7.8						H	74	+6	139	9.6						22
44	I	32	H	80	+27	145	13.8	H	73	+63	149							H	65		154	19.7						18
44	II	32	H	65		154	19.7						H	66	+45	173												11
44	III	33	H	66	+45	173												H	64	+34	200							15
45	I	37	SH	71	+35	158		H	89	+29	163							H	58	+8	197							21
46	I	31	SH	88	+48			H		+20								H	73	+9								14
47	I	37	SH	90	+22	108																	H	70	+19	124	14.9	31
48	I	34	SH	58	+66	113	29.4						H	13		234		H	69	+35	170		H	63	+20	174	15.8	55
49	I	59	H	65	+41	173	13.5	H	58	+50	172	10.3	H	60	+11	204	10.0											12
50	I	43	H	53	+68	155	12.8						H	62	+46	140	12.6											13
51	I	61	H	70	+39	225	16.4	H	24	+39	212	14.0	H	82	+41	178	10.2	H	85			11.1						16
52	I	65	H	80	+50	164	21.9						H	45	+48	345	5.8	H	58	+45	240	11.3	H	63	+64	148	10.7	41
66	I	62	H	86	+40	115	15.3						H	70	+68	137	24.0											12
N		31		31	28	29	13		16	17	15	5		11	9	10	5		19	16	16	6		8	8	7	4	31
Mean		37		73	+47	154	17.9		59	+40	154	11.0		61	+41	185	12.5		66	+33	163	12.0		64	+34	164	12.7	21

Abbreviations as in Table I.

* Nodular goiter.

Table III

CLINICAL STATUS AND THYROID FUNCTION TEST RESULTS BEFORE AND SERIALLY AFTER THERAPEUTIC DOSES OF RADIOIODINE THAT RESULTED IN MYXEDEMA. MYXEDEMA GROUP.

			Before Therapy					After Therapy																				
								6-9 Weeks					10-13 Weeks					14-23 Weeks					24 Weeks or more					
Pt.	Dose No.	Age	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	CS	TU	BMR	TC	PBI	Weeks Followed
		yrs		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%		%	%	mg%	μg%	
43	III	27	H	74	+6	139	9.6						M	15	-28	300	2.2											21
45	II	37	H	58	+8	197												SM	2	-35	551		SM	1	-30	480	1.0	103
46	II	31	H	73	+9			E		-15			M	2	-29	264		M		-30	259							141
53	I	28	H	55	+2	122		H	13		169							E	1	-22	238		SM	1	-42	279	0.7	111
54	I	37	H	67	+15	244	13.5	H	0	-11	184							E	2	-25	564		SM	3	-34	790	1.0	77
55	I	28	SH	95	+37	142		E	2	-8			E	1		310		SM			342							82
56	I	41	H	62	+21	179	10.9	H	1	+5	205							SM	1	-31	434	1.6	SM	1	-32	353	1.3	71
57	I	42	H	53	+40	181		H	4	+18	205							E	1	-20	239		M	6	-20	395	2.8	56
58	I	46	H	87	-5			E		-6			M	9	-29	226												145
59	I	30	H	81	+43	174		E		+30													SM	3	-26	334		100
62	I	31	SH	82	+25	176	16.5	E	0	-1	212												M	19	-31	403	2.6	75
63	I	35	H	54	+28	153	11.4	E	4	-7	240												M	8	-22	225	1.5	62
64	I	45	H	78	+55	100	17.8											M	1	-18	285	1.1	M					28
65	I	32	H	78	+31	102	14.7											E	13	-24	310	1.9	M	19	-22	337	2.6	30
N		14		14	14	12	7		7	9	6			4	3	4	1		7	8	9	3		9	9	9	8	14
Mean		35		71	+23	159	13.5		3	+1	203			7	-29	275	2.2		3	-26	358	1.5		7	-29	400	1.7	79

Abbreviations as in Table I.

already returned to normal. It was noted clinically that patient 37 became euthyroid six months after therapy at which time the thyroid uptake and basal metabolic rate were within normal limits. In the remission group, as a rule, the thyroid uptake at six to nine weeks was well within the normal range or below even though the patient was still hyperthyroid. Nine or more months after treatment the basal metabolic rates and thyroid uptakes were usually within the normal range, although greater variability of the tests was observed than at six months despite the stable euthyroid state. In some patients test scores fell within the hypothyroid range temporarily without clinical evidence of hypothyroidism and in two instances transient myxedema was noted. At six to nine weeks 3 per cent of patients had basal metabolic rates below −20 per cent, and 36 per cent had thyroid uptake values below 10 per cent. By the sixth post-therapy month, only the thyroid uptake of one patient and the basal metabolic rate of two patients fell below the lower limit of normal. More often than not the thyroid uptake dropped to low normal levels at six to nine weeks and rose gradually in subsequent weeks. The basal metabolic rate and cholesterol tests did not follow this pattern.

All patients of the failure group (Table II) remained hyperthyroid clinically during the entire post-therapy follow-up. However, the thyroid uptake and basal metabolic rate had dropped temporarily within or below the normal range in 19 and 18 per cent, respectively, six to nine weeks following therapy. In two instances thyroid uptake was temporarily within the hypothyroid range.

Four of ten patients in the myxedema group (Table III) were still hyperthyroid clinically six to nine weeks following therapy and none had become myxedematous. In no case was the basal metabolic rate in the myxedema range. However, the values of six of seven patients who had uptake studies six to nine weeks following therapy were within the hypothyroid range. At ten weeks and thereafter clinical myxedema began to appear and the basal metabolic rates fell to myxedema levels.

The serum cholesterol reflected, in general, the clinical status of the patients in all three groups. In those who failed to show remission of symptoms little if any change occurred in serum cholesterol level, whereas those who became euthyroid or hypothyroid showed significant elevations with therapy. In some patients of the remission group the serum cholesterol concentration rose markedly after therapy, returning to normal in later weeks; however, with two exceptions examination failed to reveal any signs or symptoms of hypothyroidism in these patients and the basal metabolic rates and thyroid uptake values were usually within normal limits.

The average thyroid uptake, basal metabolic rate, serum cholesterol and protein-bound iodine for each of the three groups at each time interval are portrayed in Figure 1A to D. The distance above and below each mean value represents the 10 to 90 percentile range, that is, 10 per cent of cases fall below the bottom and 10 per cent above the top of each vertical bar. The graphs are so constructed that the distance along the ordinate representing the normal range is exactly the same for each test. Thus they are

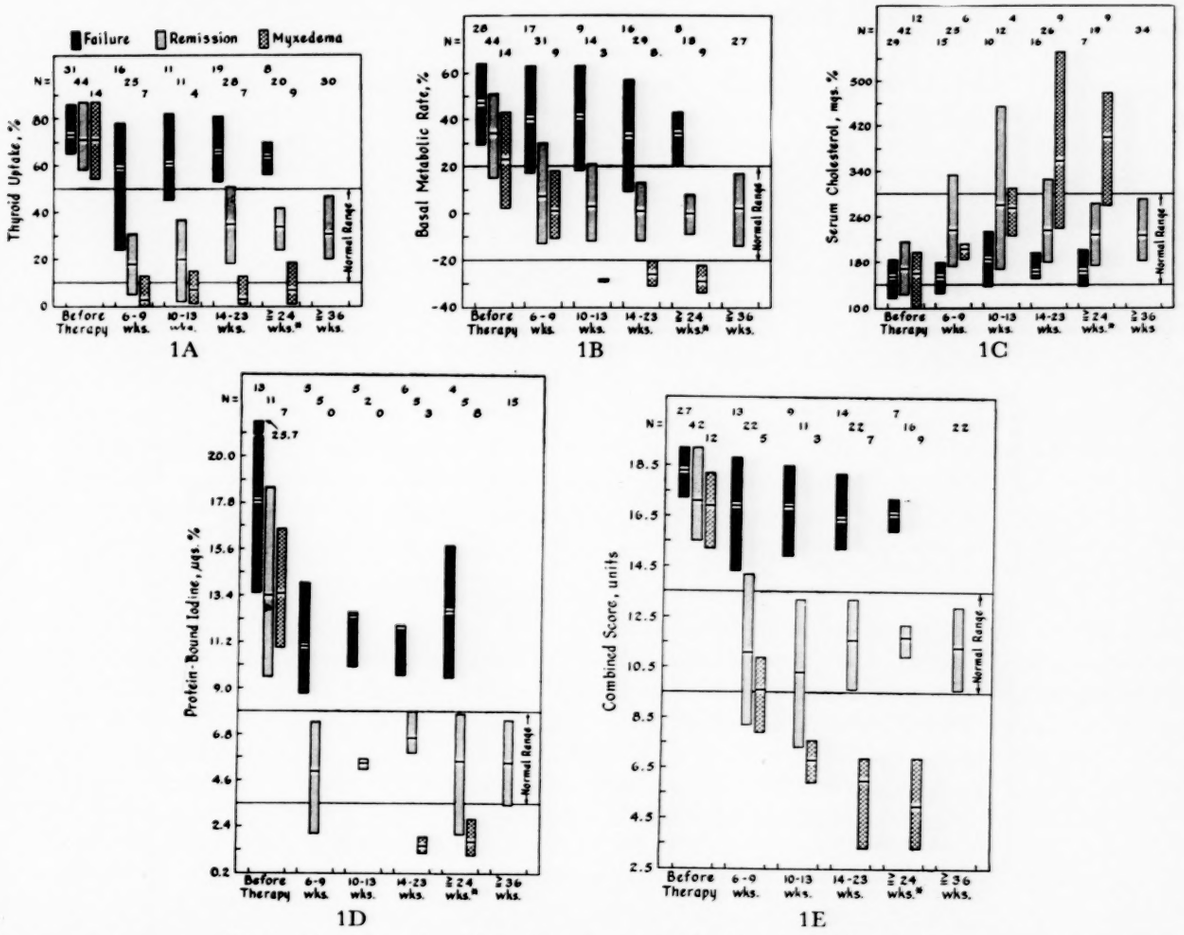

FIG. 1. Average values for four indices and combined score at each time interval. (See text.)

The horizontal lines across vertical bars represent mean values. Bottom of each vertical bar represents point below which 10 per cent of cases fall; top, point above which 10 per cent of cases fall. Number of cases upon which calculations were based are at top of graph in line with appropriate column. Code at top of Figure 1A applies to Figures 1A through 1E. Asterisks represent remission group, interval designated ≧ twenty-four weeks only, includes observations up to thirty-six weeks. A, mean thyroid uptakes before and at various intervals after radioiodine therapy, by group; B, mean basal metabolic rates before and at various intervals after radioiodine therapy, by group; C, mean serum total cholesterol concentrations before and at various intervals after radioiodine therapy, by group; D, mean protein-bound iodine concentration as determined chemically before and at various intervals after radioiodine therapy, by group; E, mean combination scores before and at various intervals after radioiodine therapy, by group.

approximately comparable from test to test, and the relative separation of the three groups by various tests may be contrasted. It is clearly apparent that for the majority of the patients the thyroid uptake as early as six to nine weeks after treatment reflected the ultimate clinical result of treatment irrespective of the clinical status at the time of testing. The mean values at each time interval fell within the appropriate range for each group. The depression of thyroid uptake in the remission group was slightly greater in the early weeks following therapy than it was later. The basal metabolic rate and serum cholesterol also reflected in general the ultimate clinical result. However, overlapping among the groups was greater, and at six to nine weeks neither delineated the myxedema group. Although based on few cases, the mean values of the chemical determination of protein-bound iodine clearly reflected the clinical result. The erratic pattern of variation is to be expected with so few cases. Thirty-six or more weeks following treatment the mean value observed with each test in the remission group approximated with remarkable consistency the corresponding mean value found in normal subjects.

The changing composition of each group with time, in respect to each of the four variables—

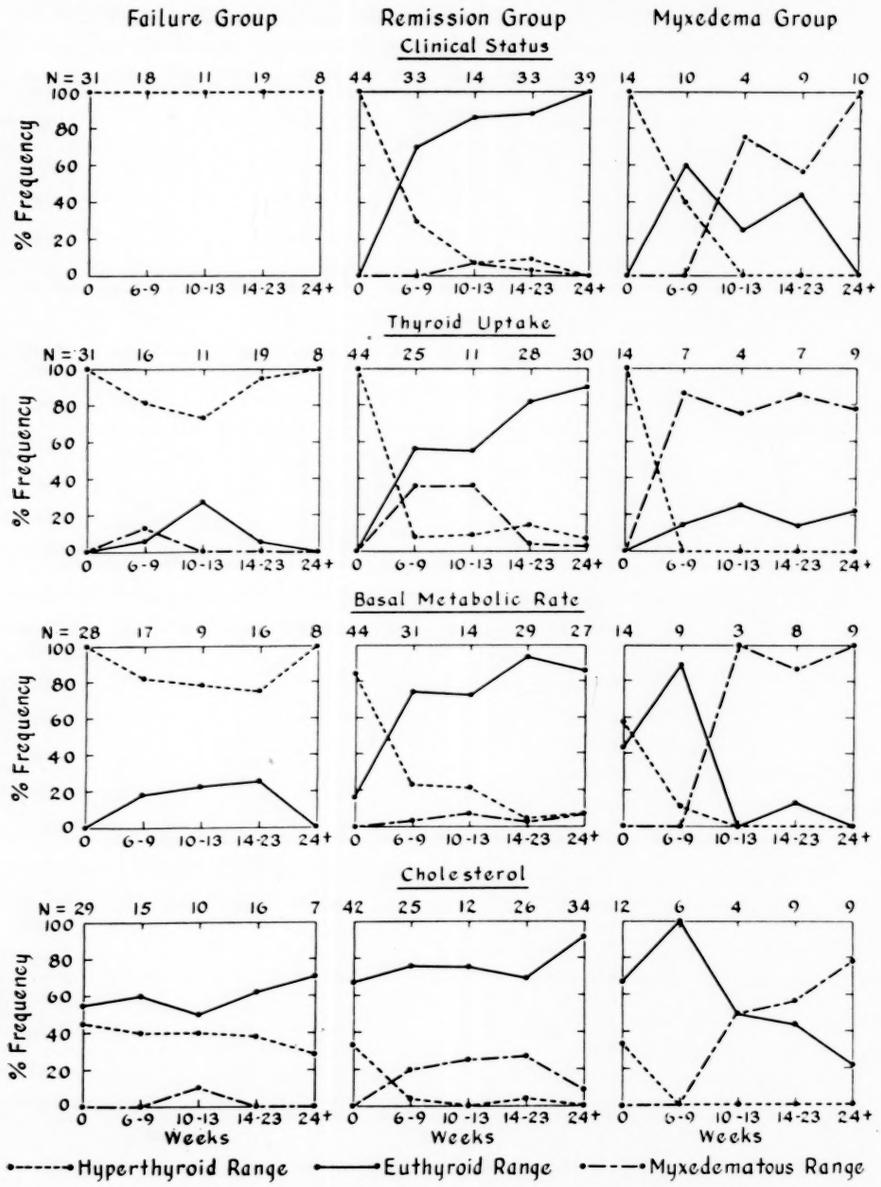

FIG. 2. Percentage of clinical appraisals or test results that were classified as hyperthyroid, euthyroid or myxedematous at various time intervals after radioiodine therapy, by variable and group. For simplicity of representation successive percentage points have been connected; however, the abscissa is not a continuous time scale. Also, although there is no specific euthyroid range of serum cholesterol concentration, normal limits were arbitrarily so designated.

clinical status, thyroid uptake, basal metabolic rate and serum cholesterol—is illustrated in Figure 2. The graphs for a given variable and a given group at a given time following therapy record the percentage of appraisals or test results that were classified as hyperthyroid, euthyroid or myxedematous.

No single measurement was sufficient for prediction but each contributed useful information. The net effect of combining the three laboratory tests for which data were sufficient and the clinical severity is of interest. A weighted combination of these four variables is plotted in Fig. 1E. The means of the groups are separated more effectively with this derived measure than with any single variable. Particularly striking

for the failure and remission groups is the progressive decrease in variability about each mean during the first nine months after treatment.

Although useful for illustrative purposes, this system of rating and combining variables is unnecessary in practice. At any given time after therapy our primary concern is whether or not to re-treat the patient who is still clinically hyperthyroid. Among our cases in no instance did hyperthyroidism recur among patients who had become euthyroid definitely by clinical criteria.

Examination of the data of Tables I, II and III led us to set up the following criteria for handling the patient six or more weeks after radioiodine treatment:

1. If the patient is hyperthyroid clinically and the thyroid uptake and basal metabolic rate are both either abnormally high or borderline (thyroid I-131 uptake 45–50, basal metabolic rate 17–20), he will very probably fail to show improvement and should be re-treated.

2. If the patient is hyperthyroid clinically and either his thyroid uptake or basal metabolic rate is definitely normal, the course is uncertain and he should be observed further.

3. If the patient is (*a*) hyperthyroid clinically and the thyroid uptake and basal metabolic rate are both definitely normal or (*b*) probably euthyroid clinically and either the thyroid uptake or basal metabolic rate is normal or (*c*) definitely euthyroid clinically, complete remission will very probably occur.

The first post-therapy observations available for each patient were utilized for classifying our cases according to these categories. Among thirty-one failures twenty-four, or 77 per cent, fell into category 1; six, or 10 per cent, fell into category 2; and one, or 3 per cent, fell into category 3. Of forty-four patient's showing eventual remission of symptoms following a single therapeutic dose, excluding those who became myxedematous, one, or 2 per cent, fell into category 1; four, or 9 per cent, into category 2; and thirty-nine, or 89 per cent, into category 3. Finally, of fifty-eight patients showing eventual remission of symptoms, including those in whom myxedema developed, one, or 2 per cent, fell into category 1; four, or 7 per cent into category 2; and fifty-three, or 91 per cent, into category 3. It is evident that none of the patients in whom myxedema developed fell into categories 1 or 2 when first seen after therapy.

When the data are classified primarily according to category as already defined we find that of twenty-five patients in category 1, twenty-four (96 per cent) belonged to the failure group. Of ten patients in category 2, six belonged to the failure group. Of fifty-four patients in category 3, only one (2 per cent) belonged to the failure group. Similar percentages were observed when the analysis was restricted to patients seen during the first six to nine weeks.

The serum cholesterol is chiefly of corroborative value. The first post-therapy cholesterol was below 150 mg. per cent in 43 per cent of the failure cases and only 4 per cent of the others. Likewise, it was over 180 mg. per cent in only 25 per cent of the failures but in 79 per cent of the others. In only one instance (4 per cent) in which remission of symptoms failed to occur was there a cholesterol over 300 mg. per cent. Twenty per cent of the remission group and 36 per cent of the myxedema group had values over 300 mg. per cent.

Insufficient data were available for us to define the value of the chemical determination of protein-bound iodine for prediction purposes. However, we might expect much from its demonstrated usefulness in diagnosis. The protein-bound iodine taken in addition to the thyroid I-131 uptake and basal metabolic rate should be particularly helpful in resolving equivocal cases, as is illustrated by patients 36 and 40 in Table I and by patients 43 II, 49 and 51 in Table II. Patient 52 in Table II demonstrates particularly well the value of information derived from all four tests.

Differences were observed between the failure and remission groups in response of gland size to therapy, as shown in Table IV. In 86 per cent of the remission group the gland had decreased in size by more than one-half, and in 54 per cent the gland was no longer palpable six to nine weeks after therapy. A similar change was observed with the myxedema group. However, among the failures only 37 per cent had a comparable decrease in gland size, and in only 5 per cent was the gland no longer palpable after six to nine weeks. With time there was little change in these percentages for the failure group until the follow-up period was extended to approximately six months, whereas in the other two groups few patients had palpable glands three months or more following therapy. The higher percentage of failures showing an appreciable reduction in gland size at six months or more reflects the elimination (earlier re-treatment) of those pa-

tients showing little or no response and retention of those with a significant partial response.

Certain differences among the response groups were also evident prior to therapy. As a group, those failing to respond were more severe clinically. Of the failures, 65 per cent were rated severe. In contrast, 30 per cent of those patients showing remission of symptoms and 14 per cent of those in whom myxedema developed were initially severely hyperthyroid. The mean basal metabolic rate of the failure group (47 $\pm$ 13 per cent) was significantly greater ($t = 4.13$, $P < .0001$) than that of the remission group (34 $\pm$ 14 per cent), which was in turn significantly greater ($t = 2.48$, $P < .02$) than that of the myxedema group (23 $\pm$ 18 per cent). The basal metabolic rate was alone among the tests in showing this pre-treatment difference between the remission and myxedema groups. A significant difference ($t = 2.39$, $P < .05$) was observed between the mean protein-bound iodine values of the failure and remission groups but not of the remission and myxedema groups. None of the other test comparisons was significantly different, although a similar trend was noted for comparisons between the failure and remission groups with the thyroid uptake and serum cholesterol tests. In general, the failure group consisted of patients who were the most severely thyrotoxic initially, and the myxedema group consisted of patients who were the least thyrotoxic initially. This experience suggests that the size of the therapy dose should be based in part upon the severity of the disease.

TABLE IV
CHANGES IN GLAND SIZE AT VARIOUS INTERVALS AFTER RADIOIODINE THERAPY

	Interval Following Therapy				
	6–9 Weeks	10–13 Weeks	14–23 Weeks	24–35 Weeks	36+ Weeks
Failure group:					
No. of cases	19	8	16	5	2
<50% decrease, % of cases	63	63	56	20	0
>50% decrease, % of cases	37	37	44	80	100
Gland not palpable, % of cases	5	12	6	20	0
Remission group:					
No. of cases	28	12	27	20	31
<50% decrease, % of cases	14	0	0	0	0
>50% decrease, % of cases	86	100	100	100	100
Gland not palpable, % of cases	54	75	93	100	100
Remission and myxedema groups:					
No. of cases	38	16	36	27	37
<50% decrease, % of cases	13	0	3	0	0
>50% decrease, % of cases	87	100	97	100	100
Gland not palpable, % of cases	50	81	89	100	100

COMMENTS

The need for reliable criteria of response to therapy is self-evident, and the subject has been considered in a variety of reports. Data are meager, however, because extended serial observations of patients after therapy are necessary before the question can be approached. Though no one apparently has heretofore presented specific prognostic criteria that could be applied with high probability of success to the individual patient, various reports have discussed and illustrated selected phases of the problem.

Rall and his coworkers[4] observed a significant relationship between the blood radioiodine concentration forty-eight hours after a therapeutic dose and the incidence of treatment failures. Inadequate treatment was usually associated with a high blood level. This probably reflects the association between severity and lack of response on the one hand and between severity and protein-bound radioiodine on the other. White and Reilly[5] observed a marked reduction in the thyroxine band of plasma, chromatographically, within a week of therapy in those patients who have a remission. Inspection of data in the tables revealed that some patients had had thyroid uptake measurements eight to sixteen weeks after treatment. In general, a low uptake at this time was correlated with subsequent remission and a high uptake with persistence of hyperthyroidism. Kirkland[6] noted that patients treated with I-131 showed the same "injury" type of thyroid uptake curve two or more months after therapy as patients receiving antithyroid drugs; he noted also that potassium thiocyanate caused a loss of thyroid radioiodine. He interpreted this as a defect in the ability of the thyroid gland to form organic iodine compounds and suggested that it might be used as a test of the response to radioiodine. Kurland, Freedberg and Fishman[7] studied euthyroid cardiac patients given a second dose of radioiodine as early as seven days following the first dose. They noted a significant decrease in

the thyroid uptake and biologic half-life with the second dose. Thyroid stimulating hormone reversed this effect. Benua and Dobyns[8] generally found normal or low thyroidal uptakes subsequent to radioiodine therapy in patients who responded well to therapy. Gland size also decreased significantly. They recorded changes in the amount of thyroidal I-131 in eight patients, seven of whom had good therapeutic responses. A break in the curve toward a more rapid rate of loss was thought to indicate a good therapeutic result; however, the one patient with poor response had a similar break in the curve.

The two most elaborate efforts to evaluate the iodine-concentrating function of the thyroid gland after treatment with radioiodine were made by Myant[9] and by Larsson and Ragnhult.[10] Myant recorded the neck-to-thigh ratio, a measure of iodine uptake, three weeks after each of thirty-six treatments given to twenty-five patients. Of twenty-eight treatments in which a drop of 30 per cent or more in the ratio was observed, 71 per cent eventually resulted in acceptable remissions whereas 29 per cent did not. Of eight treatments with less than 30 per cent decrease only one resulted in remission. In all but a few instances the ratio observed at three weeks was lower than at any later time, suggesting to the author that thyroid function is usually inhibited to a greater extent at three weeks than it is later.

Larsson and Ragnhult[10] performed serial uptake studies after radioiodine therapy in twenty-three thyrotoxic and ten euthyroid cardiac patients. The first post-therapy tracer test was performed after two to four months and the second after five to nine months. In most instances an initial depression of function occurred, followed by recovery to a level usually below that of the pre-treatment studies. Like Myant, the authors observed very low values in several patients who eventually developed myxedema and similar low values in others whose iodine-concentrating function improved subsequently and who became and remained euthyroid.

Both of these studies bring out the usefulness of estimation of the thyroid uptake within the first months after radioiodine therapy; however, the number of patients studied and the separation of patients observed were not sufficient to allow comfortable prediction in the individual case.

From our data it is apparent also that in most patients the thyroid uptake very shortly after treatment reflects the true extent of thyroid injury. In general, it can be relied upon for prediction of success or failure of therapy at a time when other tests and clinical findings may not do so. During the early post-treatment period the basal metabolic rate, cholesterol, protein-bound iodine as chemically determined and the clinical findings primarily reflect the reservoir of thyroid hormone which must be utilized before a remission of symptoms occurs and, more so, before myxedema appears. It is reasonable to expect that some basic measure of thyroid cell function *per se*, such as the thyroid uptake, would reflect the degree of radiation injury much earlier than those measures related primarily to the quantity of circulating hormone. The greatest decrease in thyroid uptake after treatment occurred at six to nine weeks, the earliest interval at which our patients were studied. Myant found a similar effect at three weeks. It seems likely that the thyroid uptake could give this information within the first week or two, given a suitable method of measuring uptake so soon after the treatment dose, since three-fifths of a typical dose is ordinarily delivered to the gland during the first week and six-sevenths during the first two weeks. The use of another isotope of iodine, such as I-132, together with a system of differential counting provides a means of solving this problem.

After its initial depression the thyroid tended to recover its function somewhat in those patients who showed remission of symptoms but did not develop myxedema. As noted by Myant,[9] this recovery phenomenon is due to one or more of three possible effects: (1) temporary injury of cells which later recover, (2) destruction of cells with compensating hyperfunction of the survivors, or (3) hyperplasia of the surviving cells. When uptake studies were made long after therapy the values were still reliable indicators of thyroid function. Although they had usually risen above the level observed shortly after treatment, they were with few exceptions still within the normal range in the remission group.

Exceptions to the general rules of change in test values following therapy will occur, as we observed in our own data. However, tracer and other measures of thyroid function give misleading results at times when used diagnostically, and the post-therapy studies should not be expected to do better.

Although the thyroid uptake was very effective in predicting the response to treatment shortly after therapy, much was gained by utilizing the information supplied by the other variables. The serum cholesterol concentration reflected the changing clinical state in any given patient surprisingly well; however, the changes were not sufficiently consistent from patient to patient for it to contribute much to a predictive index. Taking into account the clinical state, the thyroid uptake and the basal metabolic rate simultaneously gave maximal effectiveness in predicting the outcome in an individual patient. Addition of the chemical determination of protein-bound iodine would undoubtedly enhance the efficiency of prediction, as suggested by the changes observed in Figure 1D; however, our data were too meager for us to utilize it in outlining the criteria of response.

The prediction of myxedema is not of as much practical importance as the prediction of failure of response to therapy, yet the knowledge that myxedema may occur aids in management of the patient who will then be observed more frequently and carefully for appearance of symptoms and signs. It is interesting that some patients had a rather marked transient rise in serum cholesterol and some a very low basal metabolic rate and thyroid uptake comparable to values found in frank myxedema, yet they did not exhibit clinical signs or symptoms at any time. This phenomenon is probably a result of the rapidly changing state of thyroid function. Although the tests of function reflect the transient hypometabolic state, a lag in the appearance of clinical manifestations occurs and before they become apparent thyroid function has already recovered.

Presumably, early re-treatment should be attempted if on the basis of the criteria presented a treatment failure is likely. The concern that a patient might continue to improve after six to nine weeks while yet remaining a treatment failure is not supported by our data. The patients considered treatment failures who were seen several times after treatment showed no significant change in clinical severity from that observed at six to nine weeks. Also, for each of the test variables the average scores observed at fourteen or more weeks were essentially similar to those observed at six to nine weeks. Most of the small group of patients in whom there is question as to the ultimate outcome will be failures. It is probably advisable to give such patients antithyroid drugs until the uncertainty can be resolved.

SUMMARY

Sixty-six hyperthyroid patients given eighty-nine therapeutic doses of radioiodine were evaluated before and serially after therapy in regard to clinical status, thyroid uptake, basal metabolic rate, serum cholesterol and protein-bound iodine as determined chemically.

Three groups of patients—those showing failure, remission and myxedema—were distinguished by clinical criteria after adequate follow-up (average of twenty-one, seventy-seven and seventy-nine weeks, respectively). Differences in clinical severity were evident prior to treatment. Approximately two-thirds of the failure, one-third of the remission, and one-seventh of the myxedema groups were severely hyperthyroid initially. The basal metabolic rate was the only test differentiating significantly all three groups before treatment.

The response groups were clearly distinguishable by the thyroid uptake as early as six to nine weeks following therapy and at each period of observation thereafter. The basal metabolic rate was almost as effective and the cholesterol somewhat less effective in differentiating failures from remissions. Though based upon very small samples, the protein-bound iodine appeared at least as effective as the basal metabolic rate. With one exception a combination of the variables was more effective than any single measure in separating the three groups at every time interval after therapy.

Ninety-six per cent of those who were hyperthyroid clinically and whose uptake and basal metabolic rate were both either abnormally high or borderline at their first adequate post-therapy evaluation proved eventually to be treatment failures. Six of ten who were hyperthyroid clinically, but with either uptake or basal metabolic rate definitely normal, were treatment failures. Only 2 per cent of those who were (1) hyperthyroid clinically with uptake and basal metabolic rate both definitely normal, or (2) probably euthyroid clinically with either uptake or basal metabolic rate normal, or (3) definitely euthyroid clinically, were failures.

The serum cholesterol was chiefly of corroborative value.

As early as six to nine weeks, over one-half of the patients in the remission and myxedema groups but only one-twentieth of those in the

failure group had non-palpable glands. At later intervals the group differences were even more striking.

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Absorption of Radioactive Vitamin B_{12} in the Syndrome of Megaloblastic Anemia Associated with Intestinal Stricture or Anastomosis*

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In the course of investigations relating to the absorption of vitamin B_{12} from the intestinal tract two patients with megaloblastic anemia associated with intestinal lesions have been studied. Because recent clinical observations have suggested that administration of antibiotics may cause a hematologic response in certain megaloblastic anemias,[1-3] we have been prompted to study the effect of antibiotics on the absorption of vitamin B_{12} in these two patients.

Since the syndrome of megaloblastic anemia associated with intestinal lesions is rare, its clinical features as well as theories of mechanism of the anemia will be discussed, and the two cases presented.

REVIEW OF THE LITERATURE

In 1895 Faber[4] reported a case in which the clinical picture of pernicious anemia was associated with an intestinal stricture. Since then a number of similar cases have been described, and in 1929 the subject was reviewed by Meulengracht[5] who collected twenty-two cases of this syndrome from the literature. In this disease not only may the blood picture and bone marrow be indistinguishable from addisonian pernicious anemia, but subacute combined degeneration of the spinal cord also may occur, as well as glossitis and icterus. However, in about 50 per cent of these patients the gastric juice has been found to contain HCl, and intrinsic factor has been demonstrated when appropriate tests have been made.[6] Thus the etiology and pathogenesis appear to differ from addisonian pernicious anemia.

In 1929, Little, Zerfas and Trusler[7] found a characteristic clinical picture of pernicious anemia in a young man who had had several operations for intestinal fistula which had developed following acute appendicitis. This resulted in an anastomosis between jejunum and ascending colon with a blind loop of intestine and stasis of intestinal contents. It appears to be the first recorded case of macrocytic anemia following anastomosis. Additional cases of macrocytic anemia associated with either stricture or anastomosis have been reported, although it is sometimes difficult to separate the lesions.

In 1936 Butt and Watkins[8] reported eleven cases of macrocytic anemia associated with intestinal lesions, the majority being regional enteritis with obstructive features. They pointed out that any intestinal lesion which produces obstruction may be associated with macrocytic anemia. In 1939 Barker and Hummel[9] made a review of the literature, collecting forty-nine cases and adding two of their own.

The next review was made in 1949 by Cameron, Watson and Witts[10] who found a total of sixty reported cases, adding one of their own. Their analysis of these sixty-one cases in which an adequate record was available revealed that glossitis was present in thirty-three, neurologic disease in twelve, icterus in nineteen and macro-

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cytosis in all but one case. Free HCl was found in twenty-four cases, was absent in twenty, and not recorded in seventeen.

These authors noted that liver therapy had been used in twenty-seven of the sixty-one cases and that a response had occurred in twenty-two. They found that liver therapy had been less effective than in addisonian pernicious anemia but pointed out that many of the patients were extremely debilitated or had complicating lesions such as carcinoma.

In twenty-five of the sixty-one cases surgery was attempted for correction of the intestinal abnormality. Eleven of the patients died postoperatively. In eight the anemia was cured by the operation, while in six no benefit had accrued.

Of these sixty-one cases, intestinal stricture was believed to be the background of the disease in thirty-seven (in six of these the stricture was in the colon) and in twenty-four the basic abnormality was an anastomosis. Of these anastomoses fifteen were entero-enterostomies or entero-colostomies to circumvent a stricture or diseased area of bowel, and nine were gastrocolic or high jejunocolic fistulas.

In 1953 Siurala and Kaipainen[3] reported two cases of patients with moderate megaloblastic anemia, each being associated with a blind loop following intestinal anastomoses for trauma and adhesions, respectively. In each instance therapy with aureomycin or terramycin was followed by a hematopoietic response. Later both patients were operated upon to eliminate the blind loop, with resultant cure of the anemia.

Davidson[11] has made an important contribution to the subject in reporting the autopsy findings in a case of megaloblastic anemia associated with intestinal tuberculosis, in which there was subacute combined degeneration of the cord. Microscopic examination revealed, in addition to widespread tuberculosis of the upper small intestine, that the patient had a normal histologic picture of the stomach, while demyelinization of the posterior and lateral columns of the cord was present. This is the only instance in which there had been histologic documentation of spinal cord involvement in the megaloblastic anemia associated with intestinal lesions.

Until 1952 no data regarding the response to folic acid or vitamin B_{12} had been reported. Since then, however, Hall[12] has reported two cases; one of these patients responded to folic acid and the other to vitamin B_{12}. Thompson and Ungley,[13] Thomson[14] and Pearson[15] have all published case reports in which vitamin B_{12} produced a definite response, and Naish[16] cites two cases (which were not published in detail) in which folic acid was effective.

These reports comprise a total of seventy-six cases. Thus it is evident that the disease is not very common. Certainly it does not occur in all patients in whom an intestinal stricture or a blind loop is present, although it has been noted that many years may elapse after the operative procedure before anemia develops. With the decline in incidence of intestinal tuberculosis, which used to be a frequent cause of intestinal stricture, and with improvement in surgical technic, resulting in fewer instances of stagnant loops of intestine following intestinal surgery, it is probable that correspondingly fewer cases of megaloblastic anemia have occurred in recent years.

MECHANISMS INVOLVED IN THE ANEMIA

The mechanisms responsible for megaloblastic anemia associated with intestinal lesions have been the subject of much speculation. Because the gastric juice of a majority of the patients has contained free HCl, with demonstration of intrinsic factor activity in a few cases, and because surgical correction of the abnormality has resulted in cure without any other therapy in several patients, simple coincidence with addisonian pernicious anemia may be ruled out.

Until recently other mechanisms which have been considered include reduction in absorptive capacity of the small bowel such as occurs in sprue, and the elaboration of a toxin from bacterial putrefaction which interfered with proper blood formation.

In connection with the belief that a reduced absorptive surface of the intestine might be responsible it is of interest that Jackson and Linder,[17] in an extensive review of the metabolic and clinical effects of massive resection of the small bowel, state that anemia is not a feature of that situation when the absorptive surface is drastically reduced. When studies of intestinal absorption have been made, the results have shown that in most of the cases absorption, especially of glucose, has been good. However, steatorrhea was found to be present in about one-fifth of the sixty-one cases analyzed by Cameron, Watson and Witts.[10] Since megalo-

blastic anemia and steatorrhea are features of the sprue syndrome the question may properly be asked whether some of the cases may not be secondary sprue with abnormal small bowel absorption associated with the underlying lesion, the so-called "malabsorption syndrome." Under these circumstances the megaloblastic anemia would be caused by defective absorption of hematopoietic agents resulting from a defect in the intestinal mucosa. However, further analysis of cases of steatorrhea reveals that about half were associated with gastrojejunocolic, duodenocolic or high jejunocolic fistula, where steatorrhea may be expected regardless of anemia.

Since steatorrhea is not a very common feature in cases of megaloblastic anemia associated with intestinal lesions, it seems probable that a defect in the capacity of the intestinal mucosa to absorb hematopoietic agents along with fat, as one assumes to be the case in the megaloblastic anemia of sprue, is not the primary basis of the anemia.

In respect to the bacterial toxin theory it may be pointed out that the relationship of bacteria to the pathogenesis of the syndrome has been prominent in the discussions from the beginning. Faber[4] suggested in 1895 that the anemia resulted from absorption of a poison from the stagnant bowel contents. Meulengracht[19] in 1921 thought that the anemia in this disease was probably associated with infection in the bowel above the stricture. In 1924 Seyderhelm[20] produced intestinal strictures in ten dogs. In two of the ten a progressive anemia developed "which was of marked hyperchromic character." In these two dogs postmortem examination revealed marked stagnation with a luxuriant growth of bacteria throughout the small intestine above the stricture, but in the dogs which did not become anemic the bowel remained sterile even though strictures were also present.

Renshaw and his co-workers,[18] studying gastrocolic fistula, noted that macrocytic anemia developed in two of seven dogs in which it was experimentally produced. They emphasized that in this syndrome the stomach contents do not pass into the colon readily but that colonic material does pass into the stomach easily. This led to the hypothesis that the basis for the manifestations of the syndrome was contamination of the stomach and small intestine with bacteria from the colon, rather than direct passage of food from stomach to colon which would by-pass the digestive and absorbing surfaces of the small intestine.

With the advent of antibiotics a new method for studying certain relationships between bacteria and nutrition, including hematopoiesis, became available. Lichtman, Ginsburg and Watson[1] reported that aureomycin, given to four patients with pernicious anemia in whom relapse was present and to one patient with nutritional macrocytic anemia, resulted in a slow but definite hematopoietic response. Whether this could be explained by improved utilization of vitamin B_{12} or of folic acid, or by some other mechanism cannot yet be determined. In certain macrocytic anemias, presumably of nutritional origin, occurring among Africans in Kenya, Foy and Kondi[2] reported that dramatic improvement occurred after treatment with oral penicillin alone. It was speculated that the diet of these patients which was high in carbohydrate and low in animal protein might result in an abnormal bacterial flora in the small bowel.

In 1949 Cameron, Watson and Witts,[21] searching for an experimental approach to pernicious anemia, were able to produce macrocytic anemia in rats by experimentally reproducing the clinical blind loop syndrome. They produced in rats small intestinal cul-de-sacs in the upper part of the small intestine. Provided these were constructed in such a way that intestinal peristalsis would tend to keep the blind pouch filled and thus stagnant a considerable proportion of the rats surviving the operation six months or more developed macrocytic anemia. They found that the anemia responded well to folic acid but not to liver extract or vitamin B_{12}. It also responded to treatment with aureomycin.

In 1950 Toon and Wangensteen[22] confirmed these findings. They found that anemia occurred regularly after an operation designed to produce a blind loop, the anemia being macrocytic in about half the cases. However, if aureomycin was given in the diet starting at the time of the operation, the development of anemia was prevented in all instances.

These observations in rats, as well as Seyderhelm's dog experiments, and the observations at autopsy that patients with the blind loop syndrome and megaloblastic anemia have a luxuriant bacterial growth in the stagnant loop, all point to the view that intestinal bacteria may have an adverse effect on hematopoiesis.

In order to test this concept more directly we

have determined the absorption of vitamin B_{12} in the two patients to be reported, by measuring the degree of radioactivity present in the stools after oral administration of cobalt^{60}-labeled vitamin B_{12}.* This test was than repeated, first with concomitant administration of intrinsic factor, and then during the administration of aureomycin, achromycin® or neomycin.

CASE REPORTS

CASE 1. J. S., a twenty-four year old white Mexican male hospital laboratory worker was admitted to the hospital on January 28, 1954, because of easy fatigability and weakness. In January, 1952, while serving with the armed forces in Korea, he complained of the same symptoms and was found to be markedly anemic. He had glossitis at that time. On his return to the United States he was hospitalized at an Army hospital where studies revealed the presence of free hydrochloric acid, macrocytic, hyperchromic anemia, and bone marrow with some degree of megaloblastosis. Treatment with parenteral vitamin B_{12} resulted in marked symptomatic improvement and hematologic remission. The patient received vitamin B_{12} from February to August, 1952, at which time it was stopped because it was no longer considered required. During the next sixteen months he felt well but then noticed fatigue, weakness and increasing pallor. On January 25, 1954, his hemoglobin was 8.4 gm./100 cc. and bone marrow aspiration revealed megaloblastic hyperplasia, interpreted as being consistent with a nutritional macrocytic anemia.

The patient's past history revealed a series of operations at another hospital. In July, 1943, laparotomy was performed because of "intestinal obstruction." In November, 1943, exploration revealed an abscess on the left side of the abdomen, and numerous adhesions. Later in the same month the patient was re-explored and more abscess pockets on the right and left side of the abdominal cavity were opened and drained. In June, 1944, he was again operated upon because of a mass in the right lower abdominal quadrant and a fecal fistula. An appendiceal abscess was found. In April, 1945, he reentered the hospital for closure of the fecal fistula. Exploration revealed "many adhesions binding the small bowel together, with draining fecal fistulae which communicated with several openings in the underlying ileum which was bound to the abdominal wall by adhesive bands. In the left lower quadrant a small fistula communicated with two adjacent loops of sigmoid."

Physical examination at the time of the present admission showed a pale, thin white man with slight scleral icterus. The tongue was normal. Neurologic examination showed no abnormalities. There were well healed surgical scars in each lower quadrant. Free hydrochloric acid was found on gastric analysis. The icterus index was 23, falling to 9 at the time of hospital discharge. There was no bile in the urine. The Coombs test gave negative results, and there was no sickling of red blood cells. The heterophil antibody test also gave negative results. An oral glucose tolerance test was within normal limits. The stools were normal, without an excess of fat. Cephalin flocculation test gave negative results, and the thymol turbidity was 7.2 units. Serum proteins were 7.6 per cent, the albumin being 6.1 per cent and globulin 1.5 per cent. The hemoglobin was 6.4 gm./100 cc., red blood count 1.8 million, hematocrit 19.5 per cent and white blood count 3,700. Blood smear revealed moderate poikilocytosis and anisocytosis with some polychromatophilia, a few nucleated red blood cells and macrocytes. The M. C. V. was 108, M. C. H. was 35, and M. C. H. C. 30. Barium enema showed no abnormalities. X-ray of the upper gastrointestinal tract and small bowel revealed a normal esophagus, stomach and duodenum. Serial films taken at half- and one-hour intervals showed an essentially normal appearing jejunum. At one and a half hours there was a marked delay in the passage of barium which remained in the ileum for eleven and a half hours. A twenty-four-hour film showed considerable retention of barium in the pelvic portion of the ileum, with the remainder in the ascending and transverse colon.

The patient was treated with parenteral vitamin B_{12} and his reticulocyte count rose from 0.3 per cent to 17 per cent after four days of treatment. The patient noted marked symptomatic improvement and left the hospital with advice to continue vitamin B_{12} injections. Two months after the start of B_{12} therapy the red blood count was 5 million and the hemoglobin 15 gm./100 cc. (Fig. 1.) To date the patient has received 30 μg. of vitamin B_{12} intramuscularly once a month. He has been entirely well and has not had diarrhea.

Comment. Although it is not possible to reconstruct the exact anatomic arrangement of the small bowel following this patient's numerous operations, judging from the x-ray report of dilated loops of the ileum there is no doubt that he has intestinal stasis. The report of one of the operations, obtained from another hospital, revealed that the patient had fistulas communicating between loops of bowel. It is of interest that the patient had two episodes of anemia, each cured by parenteral vitamin B_{12}. On each occasion glossitis occurred, and mild icterus was noted when he was under our observation. Free HCl was demonstrated on two occasions in the gastric secretions. When vitamin B_{12} therapy was stopped after the first episode of anemia, sixteen months went by before relapse. In summary, most of the symptoms and signs of pernicious anemia, as well as characteristic laboratory findings, were present in this patient with

* Kindly provided by Dr. Charles Rosenblum, Merck and Co., Rahway, N. J.

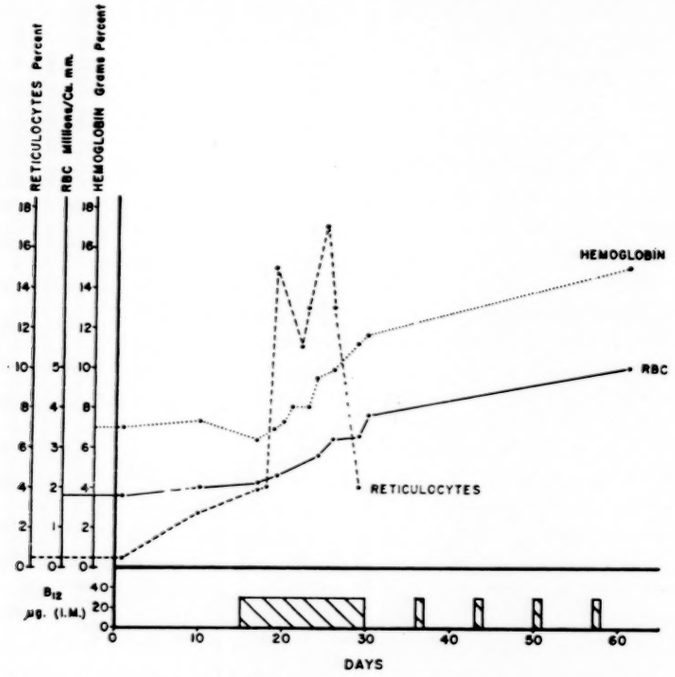

FIG. 1. Case I. Graph showing hematopoietic response to vitamin B_{12}.

evidence of intestinal stasis associated with adhesions and intestinal fistulas.

CASE II. W. M., a sixty year old man with extensive diverticulosis involving the stomach and small intestine, as well as the urinary bladder and urethra, first had gastrointestinal symptoms in 1942, consisting of attacks of diarrhea alternating with constipation. He had several attacks of small bowel obstruction, thought to be caused by jejunal diverticulitis, treated medically. In 1949 perforation of a jejunal diverticulum occurred and volvulus of the terminal ileum and cecum was encountered at operation. Resection of 2 feet of the ileum with the cecum was performed, with end-to-side anastomosis of ileum with hepatic flexure. This resulted in a blind pouch of ascending colon.

In January, 1952, the patient entered the hospital for transurethral resection of the prostate gland. On routine blood count macrocytic anemia with anisocytosis and poikilocytosis was noted for the first time. The red cell count was 2.49 million, the hemoglobin was 8.0 gm./100 cc. and the hematocrit 27 per cent. The M. C. V. was 108, M. C. H. was 32 and M. C. H. C. 30. Examination of the bone marrow demonstrated megaloblastosis and erythrocytic hyperplasia consistent with macrocytic anemia. Gastric analysis demonstrated free HCl in the stomach. Stool examination did not show increased fat. Serum proteins were normal. Treatment with vitamin B_{12} parenterally resulted in a hematologic response with a reticulocyte rise to 6 per cent. (Fig. 2.) After sixteen days of vitamin B_{12} therapy a repeat bone marrow study demonstrated increased maturation of erythrocytes with no megaloblastosis. Three months later the hemoglobin had risen to 13.8 gm./100 cc. and the hematocrit to 42 per cent. One month before hospital discharge the patient was given folic acid, without further effect on blood cell count.

Upon discharge the patient ceased taking vitamin B_{12} but continued to take folic acid orally. In May, 1954, he was readmitted with recurrent diarrhea, pallor, weakness and a loss of 25 pounds. The patient appeared poorly nourished and chronically ill. The skin showed slightly brownish pigmentation. The tongue was normal. There was slight edema of ankles, and moderate clubbing of fingers and toes. The red cell count was 3.0 million, hemoglobin 12.0 gm./100 cc. and hematocrit 33 per cent. The M. C. V. was 110, M. C. H. 40 and M. C. H. C. 36. An oral glucose tolerance curve was normal. All liver function tests were normal. The stools were negative for blood and parasites. The total serum protein was 5.4 per cent with albumin 3.3 per cent and globulin 2.1 per cent. Barium enema showed that the ileocolostomy was at the hepatic flexure with the blind loop of ascending colon below it. The remainder of the colon was normal. In roentgenograms, the upper gastrointestinal series and small bowel series demonstrated diverticulosis of the stomach, duodenum and small intestine with disordered motor function manifested by dilated loops of bowel and segmentation of the barium meal.

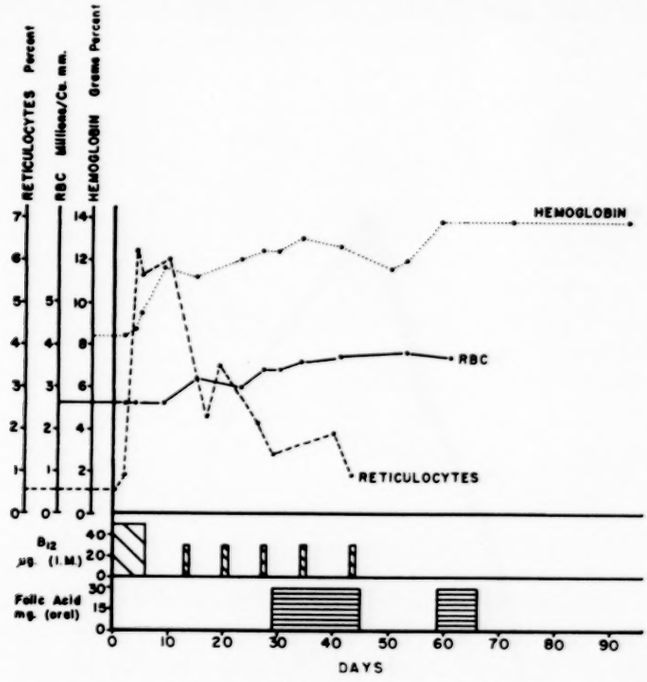

FIG. 2. Case II. Graph showing hematopoietic response to vitamin B_{12}.

The administration of folic acid was stopped and vitamin B_{12} therapy, 30 µg. intramuscularly daily, was begun. Within one week the reticulocyte count rose from a baseline of 1.4 per cent to 4.8 per cent at the end of two weeks, eventually falling to 1.2 per cent. The red blood count rose to 3.8 million with hemoglobin of 11.6 gm./100 cc. and hematocrit 37 per cent. Administration of aureomycin resulted in marked improvement in the patient's diarrhea.

Comment. Although this patient did not have an intestinal stricture, there was a cul-de-sac consisting of the ascending colon below the anastomosis of ileum to transverse colon. However, a more important factor in respect to intestinal stasis of fecal material was probably the numerous diverticula throughout the small bowel.

The macrocytic anemia and megaloblastic bone marrow were shown to respond to vitamin B_{12} alone, but not to folic acid. When B_{12} therapy was stopped, a mild megaloblastic anemia again developed despite continued folic acid administration. As will be described later, intestinal absorption of radioactive vitamin B_{12} was deficient, and the addition of intrinsic factor did not result in increased absorption as would have been the case if he had had addisonian pernicious anemia. Furthermore, there was free hydrochloric acid in the stomach. Although the patient had mild diarrhea and a small bowel "deficiency pattern," there was no increase of fat in the stools, nor did he have a flat glucose tolerance test. Thus the sprue syndrome need not be considered.

METHOD OF DETERMINING VITAMIN B_{12} ABSORPTION

In 1952 Heinle and his coworkers[23] demonstrated that patients with pernicious anemia failed to absorb cobalt^{60}-labeled vitamin B_{12}. These workers noted that it was possible to recover in the stools most of the administered radioactivity contained in a dose of 0.5 µg. of cobalt^{60}-labeled vitamin B_{12} over a four- or five-day period following its administration. When a source of intrinsic factor was given with the test dose, the fecal excretion of radioactive vitamin B_{12} decreased to around 30 per cent. This is interpreted as meaning that 70 per cent was absorbed. Swendseid, Halsted and Libby[24] showed that patients who had had a total gastrectomy were similar in this respect to patients with pernicious anemia. They were able to recover nearly 100 per cent of the administered radioactivity in such patients. If a source of intrinsic factor was given with the test dose only 10 to 30 per cent of the radioactivity was recovered in the stools. These findings indicate that intrinsic factor is necessary for the absorption of vitamin B_{12}, and also that the stomach is the principal if not the only site of secretion of intrinsic factor. Halsted, Gasster and Drenick[25] reported that normal persons excreted an

average of 32 per cent of a test dose of 0.5 μg., the smallest amount excreted being 19 per cent and the greatest amount 57 per cent. Callender, Turnbull and Wakisaka[26] found that ten normal persons excreted almost identical amounts, the average being 31 per cent, with lower and upper limits of 14 and 45 per cent, respectively. Further studies in this laboratory indicate that when fecal excretion tests with 0.5 μg. of radioactive vitamin B_{12} are made in the same person under similar conditions, the greatest variation in fecal excretion between the two tests was 18 per cent. (Table I.) Within this limitation, the test may be considered quantitative. In other words, when the same dose is repeated in a patient, a change in fecal excretion of at least 18 per cent must occur before one can attribute a change in absorption to the effect of an agent used in the second test for the purpose of influencing absorption, such as intrinsic factor or an antibiotic.

TABLE I
PER CENT FECAL EXCRETION OF 0.5 μG. $CO^{60}B_{12}$ IN PATIENTS WITH REPEATED TESTS

Case No.	Diagnosis	Test No. 1	Test No. 2	Per cent Difference
1	Normal	53	48	5
2	Normal	27	25	2
3	Normal	32	29	2
4	Normal	27	30	5
5	Normal	32	30	2
6	Normal	26	40	14
7	Enteritis	53	80	17
8	Sprue	58	44	14
9	Sprue	74	86	12
10	Pancreatitis	76	72	4
11	Enteritis	77	82	5
12	Blind loop	59	77	18
13	Jejunal diverticula	84	98	14
14	Enteritis	64	71	7

Details of the method of carrying out the test in our laboratory have been described elsewhere.[25]*

In the present study 0.5 μg. of cobalt[60]-labeled vitamin B_{12} with a specific activity of 220 μc. per milligram was administered to each of the two patients whose case reports have been presented. The test was then performed with a source of intrinsic factor administered with the test dose. Then, either aureomycin or achromycin was given in doses of 2 gm. daily for three days, at which time the test dose of radioactive vitamin B_{12} was again administered. After collecting stools until radioactivity no longer appeared (usually four days) the test dose was again repeated while continuing the antibiotic. This was given in amounts of 1 gm. daily throughout the period of stool collections after the first three days. From ten to fourteen days of antibiotic administration were required to complete the two tests. At a later date the experiment was repeated during administration of neomycin in doses of 6 gm. daily.

TABLE II
EFFECT OF INTRINSIC FACTOR AND ANTIBIOTICS ON FECAL EXCRETION OF 0.5 μG. $CO^{60}B_{12}$ IN TWO PATIENTS WITH MEGALOBLASTIC ANEMIA ASSOCIATED WITH INTESTINAL STASIS

Date (1954)	Test Dose of 0.5 μg. $Co^{60}B_{12}$	Fecal Excretion %
	Patient J. S.	
9/9	Given alone	59
2/16	Given alone	77
2/25	With intrinsic factor	92
3/19	With aureomycin	25
3/26	With aureomycin	15
9/14	Given alone	72
9/24	With neomycin	63
	Patient W. M.	
6/4	Given alone	98
6/8	With intrinsic factor	95
6/14	With aureomycin	44
6/19	With aureomycin	65
7/26	Given alone	84
8/4	With achromycin	63
8/10	With achromycin	60
9/13	Given alone	91
10/12	With neomycin	93
10/22	With neomycin	92
11/8	With gantrisin	97

* Two other methods for determining intestinal absorption of radioactive vitamin B_{12} have been reported. In the first, an injection of 1,000 μg. of nonradioactive B_{12} is given two hours after oral administration of 2 μg. of cobalt[60]-B_{12}. This results in a "flushing out" of vitamin B_{12} through the kidneys so that radioactivity can be detected in the urine in the succeeding twenty-four hours if absorption has occurred.[27] In the other method surface scintillation measurements are made of the uptake of radioactive vitamin B_{12} by the liver. If radioactivity can be detected over the liver several days after an orally administered test dose, it can be assumed that absorption has occurred.[28]

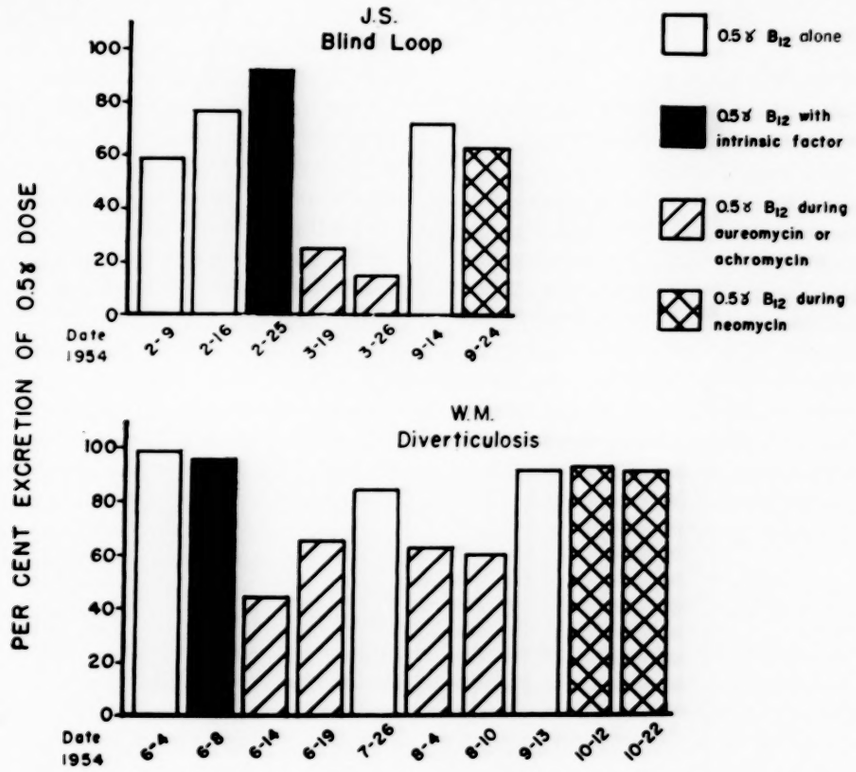

FIG. 3. Effect of intrinsic factor and of antibiotics on fecal excretion of cobalt^{60}-labeled vitamin B_{12} in two patients with megaloblastic anemia associated with intestinal stasis.

RESULTS

The fecal excretion of radioactive vitamin B_{12} expressed in per cent of the test dose of 0.5 μg. is recorded in Table II for each patient and is represented graphically in Figure 3.

In determining differences in fecal excretion from test to test we have compared the tests in which the least difference occurred, instead of averaging them when more than one was performed. The tests have been divided into the following three categories: given alone, with intrinsic factor and with antibiotic.

In both patients the effect of intrinsic factor was tested; 50 mg. of a hog mucosa extract* was mixed with the test dose of cobalt$^{60}B_{12}$. In neither of the patients did this cause any decrease in fecal excretion. In fact excretion was considerably greater in patient J. S.

The effect of antibiotics was tested in several experiments. In patient J. S. excretion of 59 and 77 per cent occurred during two tests, when the test dose was administered alone. During aureomycin administration the excretion was only 25 and 15 per cent in two tests performed six days apart. Six months later, when the patient was entirely well and had a normal blood count, the fecal excretion was again measured and found to be 72 per cent. The decreased fecal excretion during administration of this antibiotic was very marked, there being a difference of at least 34 per cent. The results of the experiment with neomycin were quite different in that fecal excretion was essentially the same as when cobalt$^{60}B_{12}$ was given alone, 63 per cent.

In patient W. M. three experiments were carried out. In the first, 98 per cent of the test dose was excreted; then, with aureomycin, 44 and 65 per cent was excreted in two consecutive tests. This represents a decrease of at least 33 per cent. In the second experiment achromycin was used and the change was less marked (a 21 per cent decrease in fecal excretion). In the third experiment, performed two months later, neomycin was used, resulting in no change in fecal excretion over what was obtained when cobalt^{60}-B_{12} was administered alone. Excretion

* Kindly supplied by Dr. R. W. Heinle of Upjohn Co., Kalamazoo, Michigan.

during neomycin administration was 93 and 92 per cent in two tests.

Somewhat later B_{12} absorption was tested during administration of a sulfonamide, gantrisin.® This had no effect, fecal excretion being 97 per cent.

Because the patient had diarrhea, which was ameliorated by both aureomycin and achromycin (but not by neomycin), it was thought that the improved absorption during these experimental periods might have been caused by a slowing of the intestinal rate. However, while the patient was given gantrisin the diarrhea also disappeared entirely, yet fecal excretion of cobalt$^{60}B_{12}$ was just as high as when the test dose was given alone, at which time moderate diarrhea was present. This indicates that diarrhea of itself was not the reason for the high excretion.

OBSERVATIONS

In both cases it can be presumed that an abnormal small intestinal bacterial flora was present since there is evidence from both autopsy observation and animal experiments that such is the case in this syndrome. The impaired absorption of radioactive vitamin B_{12} might be explained by bacteria absorbing B_{12}, and thus denying it to the body. Under these circumstances the radioactivity recovered would be largely contained in the bacteria passed out in the feces. Certain strains of Escherichia coli have a marked avidity for B_{12} even though it may not be a growth requirement for these strains.[29]

Present clinical evidence indicates that megaloblastic anemia associated with intestinal stricture or anastomosis is often the result of vitamin B_{12} deficiency since several cases have recently been reported responding to vitamin B_{12} therapy. The pronounced increase in absorption of vitamin B_{12} which occurred when aureomycin was administered supports the previous clinical and experimental evidence that the fundamental mechanism in the syndrome of megaloblastic anemia associated with intestinal stasis is bacterial interference with normal utilization of vitamin B_{12}. The fact that a marked hematologic response occurred in Siurala and Kaipainen's[3] two patients who were treated only with aureomycin provides additional support for this concept. Krevans, Conley and Sachs[30] have reported the case of a patient who had megaloblastic anemia with subacute combined degeneration of the cord associated with multiple jejunal diverticula. There was normal gastric acidity. No operation had been performed, unlike the case of our patient with diverticulosis. As in our patient, these authors also found that vitamin B_{12} absorption was markedly impaired, and administration of aureomycin was followed by a return of B_{12} absorption to normal.

Whether or not folic acid enters the picture is not yet clear. However, the complex metabolic inter-relationships between it and vitamin B_{12} make it possible that there is some disturbance in the utilization or absorption of this substance as well.

To summarize the results to date of the effect of antibiotics on absorption of radioactive vitamin B_{12} in this syndrome, there was an increased absorption when aureomycin or achromycin was given in the two cases reported herein. In the case reported by Krevans et al.[30] administration of aureomycin resulted in normal absorption. In both of our cases there was no increased absorption when neomycin was given. Doscherholmen and Hagen[32] have recently reported another case of megaloblastic anemia associated with a blind loop in which neomycin did not result in any increase in vitamin B_{12} absorption. This difference in the effect of aureomycin and of neomycin is of great interest, but no adequate explanation can yet be given.

There are certain similarities between the megaloblastic anemia associated with intestinal stricture or anastomosis, and the megaloblastic anemia of Diphyllobothrium latum infestation. In both syndromes the clinical and hematologic picture is identical with addisonian pernicious anemia. In both, free HCl and intrinsic factor can be demonstrated in the gastric juice. The fish tapeworm anemia may be cured by getting rid of the worm, just as intestinal macrocytic anemia may be relieved by surgical correction of the lesion causing stasis and bacterial growth or, apparently, by treatment with certain antibiotics. Extracts of the fish tapeworm have been shown to contain large amounts of vitamin B_{12}, and injections of worm extract will induce a remission in addisonian pernicious anemia, as well as in fish tapeworm anemia.[31] Thus it has been postulated that the worm consumes vitamin B_{12} from the patient's diet, producing B_{12} deficiency in the patient. Just as an abnormal small intestinal bacterial flora does not always result in B_{12} deficiency with megaloblastic anemia, so also is the case in D. latum infestation. In Finland about 20 per cent of the population are infested yet only in about one in

3,000 does anemia develop. Numerous factors must enter into the picture in each disease. In megaloblastic anemia associated with intestinal stasis the type and number of bacteria in the stagnant area and their needs for B_{12} appear to be a fundamental factor. The amount of vitamin B_{12} stored in the body, the duration of bacterial stasis on the one hand or fish tapeworm infestation on the other, and perhaps the patients' dietary habits all seem to be important factors in the development of anemia in both conditions.

A plausible explanation for the increased absorption of vitamin B_{12} and for the hematologic response in megaloblastic anemia which may occur with aureomycin administration is that bacterial competition for hematopoietic agents is decreased. However, alternative possibilities exist. The antibiotic might exert its action by changing the flora in such a way that increased synthesis of hematopoietic material occurs. Certain organisms might be destroyed which produced a hemolytic toxin or in some other way antagonized hematopoiesis. Some antibiotics might have a specific hematopoietic action, although there is not evidence for this. The fact that neomycin did not result in any increased absorption of vitamin B_{12} in our two cases or in the one other case recently reported[32] suggests that the mechanism is more complex than simple bacterial competition.

Patients with other small intestinal disorders, such as regional enteritis and sprue, have been shown by us and by others to have a considerably decreased absorption of radioactive vitamin B_{12}.[33,34] Although it is quite likely that an abnormal bacterial flora exists in these cases, administration of aureomycin did not result in a significantly increased absorption in the patients studied by us. This fact suggests that in patients with small intestinal disease, but without an element of obstruction, the defect may be primarily an inability of the intestinal mucosa to absorb nutrient materials normally, rather than bacterial interference with utilization of hematopoietic agents. Further study is required before this matter can be further clarified.

SUMMARY

1. The literature concerned with the subject of megaloblastic anemia occurring in certain individuals with intestinal lesions is reviewed, and two additional cases of this syndrome are reported.

2. The pathogenesis of the anemia is discussed. Factors favoring the development of an abnormal bacterial flora in the small intestine are a common denominator of the syndrome. Among such factors are an intestinal stricture or an anastomosis, resulting in intestinal stasis. Gastrocolic fistula also appears to result in bacterial contamination of the small intestine, and may be associated with macrocytic anemia.

3. The absorption of radioactive vitamin B_{12} was determined in the two cases which are reported and was found to be significantly impaired in each. Administration of intrinsic factor had no effect but the administration of aureomycin or achromycin resulted in markedly increased absorption. However, there was no increase when neomycin was given.

4. These studies provide additional support for the concept that abnormal bacterial growth in the small intestine may result in impaired utilization of vitamin B_{12}, with the development of megaloblastic anemia in some instances.

5. The exact mechanism whereby intestinal bacteria may affect hematopoiesis adversely, and certain antibiotics may favorably influence it, is not yet clear.

Acknowledgment: The authors wish to express their appreciation to Mrs. Elizabeth Hvolboll for technical assistance in this investigation and to Miss Margaret Cameron for help in preparing the manuscript.

ADDENDUM

Since this paper was written we have had the opportunity to study a third patient with megaloblastic anemia associated with intestinal stasis resulting from surgical procedures. A nineteen year old white male had an appendectomy at the age of ten followed by intestinal obstruction requiring laparotomy. He had intermittent abdominal cramps until an intestinal resection was performed at the age of fifteen. The exact nature of the anastomosis could not be ascertained. The patient had no more gastrointestinal symptoms after the third operation and there was no diarrhea or other evidence of malabsorption. Three years later, however, moderate megaloblastic anemia developed with combined system disease. Both responded completely to parenteral vitamin B_{12} therapy. Administration of 0.5 μg. of cobalt^{60}-B_{12} resulted in fecal excretion of 91 per cent. When achromycin was administered, 2 gm. daily for three days, only 25 per cent of the same test dose was excreted, indicating normal absorption.

Two weeks after achromycin was discontinued the test dose was again given with intrinsic factor, with recovery of 73 per cent in the stools.

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Pulmonary Stenosis with Intact Ventricular Septum*

Correlation of Clinical and Physiologic Data, with Review of Operative Results

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PULMONARY stenosis can be defined either anatomically, by visualizing a constriction in the right ventricular outflow tract, or physiologically, by demonstrating the existence of a pressure gradient between the right ventricle and the pulmonary artery. The most common occurrence of pulmonary stenosis, thus defined, is as a component of the tetralogy of Fallot which includes pulmonary stenosis, right ventricular hypertrophy, ventricular septal defect and an overriding aorta. Operative technics[1,2] for "shunt procedures" have afforded symptomatic relief for patients with this condition during the past nine years.

The present discussion is limited to pulmonary stenosis with right ventricular hypertrophy but without ventricular defect or overriding aorta. This entity, often referred to as "isolated" or "pure" pulmonary stenosis, has only recently been recognized as one of the more common of the congenital heart lesions.[3–6] The experience of Brock[7] and Blalock,[8] as well as certain hemodynamic considerations, make the "shunt procedure" contraindicated in this condition. Brock[9] devised the first successful operation for the direct relief of right ventricular obstruction by pulmonary valvotomy. Campbell,[10] Lurie,[11] Bing[12] and Kirklin,[13] reviewing their operative results with Brock's transventricular approach, found that improvement in physiologic terms did not invariably accompany the good clinical results. Blount et al.[14] more recently have proposed another surgical approach achieving physiologic as well as clinical postoperative improvement.

Indications for operation have been variously defined by different authors. Physiologic data have been primarily considered as indications for operation by some[7,13,14] whereas clinical symptomatology has been stressed by others.[15] Another group of authors suggests that the mere presence of the lesion may be sufficient indication for surgical intervention.[16]

We propose in the present paper to reemphasize the clinical findings, to correlate these with the physiologic data, and to describe some of our operative results. A clinical method for assay of the severity of the disease will be described and our indications for surgical intervention will be outlined.

MATERIAL AND METHODS

Fifty patients with pulmonary stenosis admitted to the Children's Medical Center in the five-year period from 1949 to 1954 provided the material for this report. There were twenty-five boys and twenty-five girls. They ranged in age at the time of admission from three months to twenty-one years, although only two were over fifteen years. The average age was eight years and three months. They were all studied by the conventional clinical and electrocardiographic methods. Radiologic and fluoroscopic examinations were performed in all but two patients. These two infants were critically ill and died before x-ray examinations could be obtained.

Cardiac catheterization was performed under morphine and barbiturate sedation by the usual

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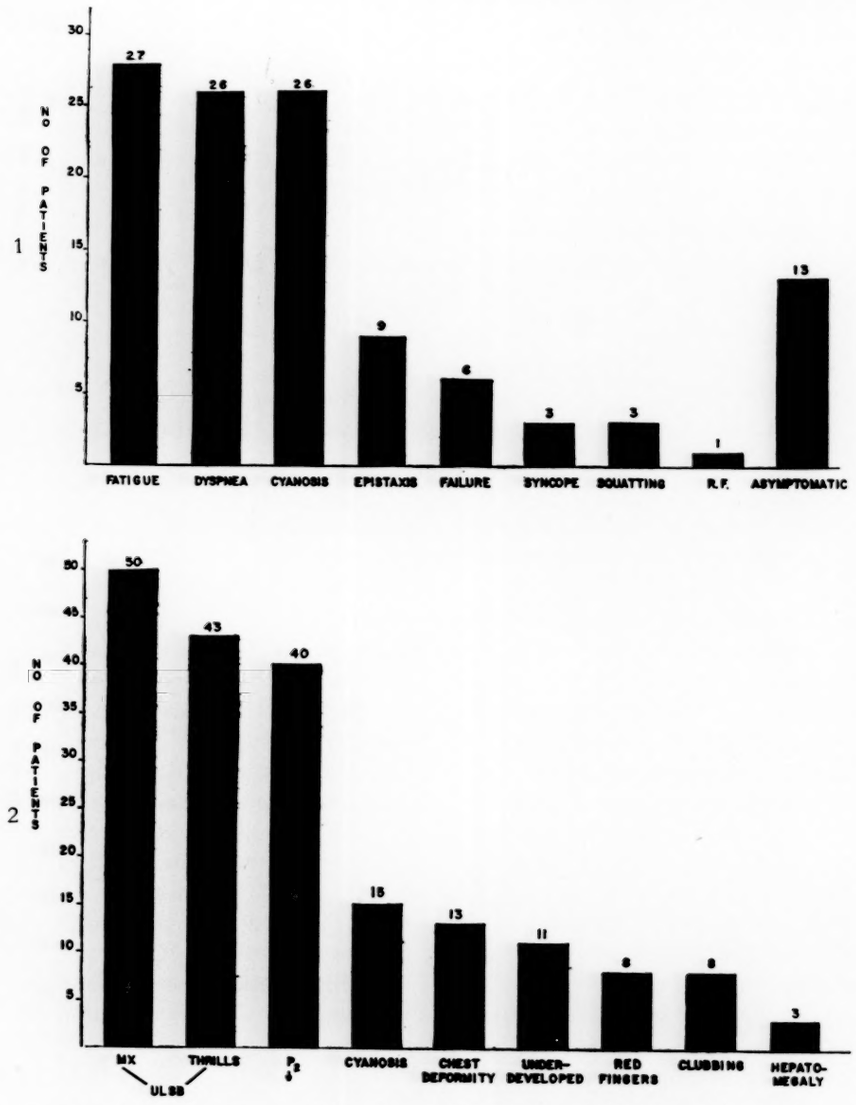

FIG. 1. Symptomatology of fifty patients. R. F., rheumatic fever.

FIG. 2. Physical examination of fifty patients. Mx, murmur; ULSB, upper left sternal border; P_2, diminished pulmonary second sound.

technic in forty-five patients. Gas analyses of the blood were made by the Van Slyke and Neill method. Pressures were obtained by the Sanborn electromanometer and tracings were registered on a Sanborn Poly-Viso apparatus. Zero point was assumed at 6 to 10 cm. from the back, depending on the diameter of the chest. Oxygen consumption was determined directly, whenever possible, by collection of expired air in a Douglas bag and use of a Pauling oxygen meter and Tissot spirometer. When direct measurement was not possible the consumption was assumed to be 170 cc./min./M^2 for children over two years and 200 cc./min./M^2 for children under two years. The cardiac output was calculated on the basis of the Fick principle. Valve orifice area was determined by the Gorlin formula.[17]

In all but two of the catheterized patients a gradient across the pulmonary outflow tract was demonstrated during the procedure. In the remaining two, right ventricular hypertension was found but the catheter could not be passed into the pulmonary artery; at operation in these two subjects a low pulmonary artery pressure was encountered. The absence of an interventricular septal defect was assumed in these forty-five patients because of the finding of a systolic peak pressure difference between the right ventricle and the systemic artery of at least 24 mm. Hg and by the failure to demonstrate a left-to-right shunt at

the ventricular level. If arterial unsaturation was encountered under these conditions, it was assumed to be due to a right-to-left shunt at the atrial level. The presence or absence of such a shunt did not seem to alter the general characteristics of the disease, hence we feel justified in discussing the two groups together. We have thus disregarded the conventional practice

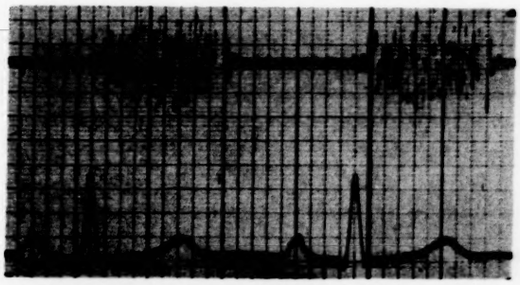

FIG. 3. Typical phonocardiogram of the "stenotic systolic murmur" in pulmonary stenosis; second left interspace; logarithmic microphone; amplification five.

of separating cyanotic from non-cyanotic patients—a view adopted also by Kirklin,[13] Campbell[18] and others in more recent articles.

The preoperative diagnosis was based on angiocardiography in two cases. The absence of a ventricular septal defect of significant size was assumed on the basis of the angiogram and proved by the postoperative pressure difference between the right ventricle and the systemic artery, as well as the absence of an intracardiac shunt. In both these patients postoperative catheterizations demonstrated a residual gradient across the pulmonary valve.

In the remaining three instances the diagnosis of pulmonary stenosis with intact ventricular septum was made at autopsy.

Operation by the Brock procedure was performed in twenty-one of these fifty patients. Postoperative catheterization data were obtained in eight.

OBSERVATIONS

Clinical Findings. The principal symptoms of our patients are presented in Figure 1. Cyanosis, dyspnea and fatigue were observed with about equal frequency. Epistaxis, syncope and squatting were noted in only a small number of the patients. Three infants were first brought to the hospital with congestive failure. Only one patient had a past history of rheumatic fever. Thirteen children were completely asymptomatic.

Analyzing the histories in some detail, one is impressed with the relatively severe and persistent nature of the dyspnea and the fatigue, in contrast to the mild and often transient cyanosis. Relatively few patients, principally those with the most severe disease, showed symptoms during the first year of life. The complaints of the majority of patients started between the ages of one and five years.

Figure 2 summarizes the findings on physical examination. A rough, long, systolic murmur,

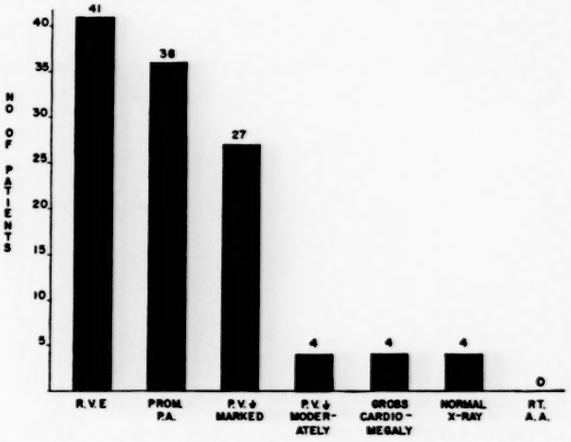

FIG. 4. Summary of radiologic findings in forty-eight, cases. R.V.E., right ventricular enlargement; Prom. P.A., prominent main pulmonary artery segment; P.V., diminution of pulmonary vasculature; Rt. A.A., right aortic arch.

maximal in intensity at the upper left sternal border, was heard in all instances. A phonocardiogram illustrating the high intensity, medium frequency, diamond shape of the murmur is presented in Figure 3. The murmur characteristically transmitted very well to the entire precordium, the neck and the back. It was frequently accompanied by a thrill, which was felt at the upper left sternal border and in the suprasternal notch.

The second sound at the pulmonic area was usually but not invariably diminished in intensity. It was frequently better heard at the lower left sternal border but was not split when maximal at that area. Splitting of the second sound, frequently observed in normal individuals,[19] was occasionally heard but only in the mild types of stenosis.

Only fifteen of the patients were noted to be cyanotic on physical examination, as contrasted with the twenty-six who had given a history of cyanosis. Thirteen patients had a mild bulge of the left side of the thorax. Only eleven were found to be physically underdeveloped (below the ten percentile in height or weight).

Redness or clubbing of the fingers was infrequent. Significant enlargement of the liver was

5A 5B 6

FIG. 5. A, postero-anterior radiogram of patient with severe pulmonary stenosis; B, left anterior oblique view of same patient.

FIG. 6. Postero-anterior radiogram of an infant with maximal pulmonary stenosis and congestive failure.

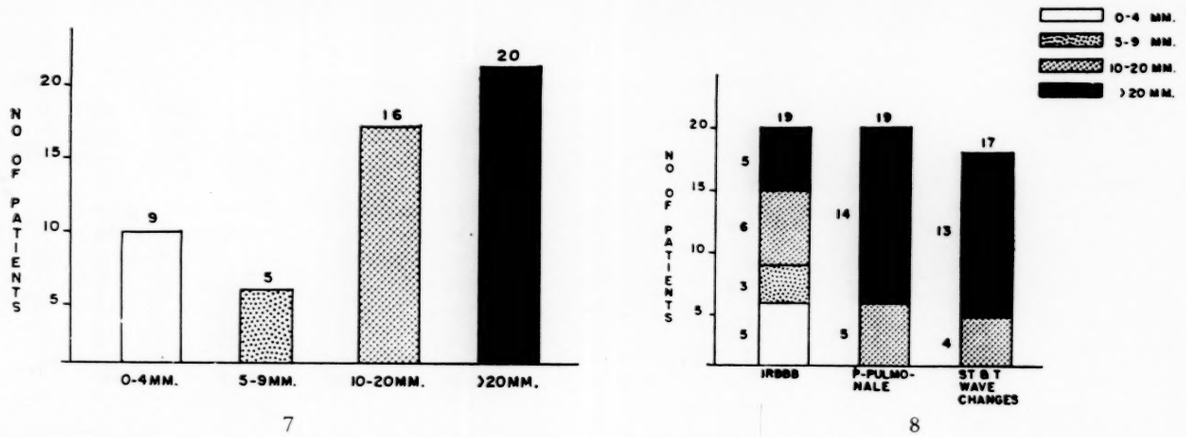

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FIG. 7. Maximal height in millimeters of right ventricular potentials (see text) in electrocardiograms of fifty patients.

FIG. 8. Right ventricular potential in millimeters related to incomplete right bundle branch block, p-pulmonale and severe right-sided ST-T wave changes.

observed in three infants with severe, pinhole stenosis and congestive failure.

Diastolic murmurs were heard in only five patients. In three of these the murmur was rumbling in character and heard early or in mid-diastole; two of these patients had a left-to-right shunt at the atrial level and one had very severe pulmonary stenosis. In a fourth patient a blowing diastolic murmur was heard immediately following the second sound, and thought to be due to the presence of rheumatic aortic regurgitation. The fifth patient—one with extremely severe disease—had two types of diastolic murmurs; one was rumbling in mid-diastole at the apex and the other was a blowing murmur along the left sternal border.

The cardiac impulse was maximal at the lower left sternal border or the xiphoid process, rather than at the apex.[19] A heaving impulse at this point was felt in the patients with severe stenosis. This type of thrust contrasted markedly with the tumultuous, hyperactive beat so commonly observed in patients with large left-to-right shunts.

Figure 4 tabulates the radiologic findings in forty-eight patients. Some degree of right ventricular enlargement, observed best in the left anterior oblique position, was present in the great majority of patients. The main pulmonary artery segment was prominent in thirty-six cases, all but one of whom proved to have a valvular type stenosis. The pulmonary vasculature was evaluated on the basis of the radiograms, rather than at fluoroscopy. It was found to be markedly diminished in twenty-seven and moderately diminished in four. Extreme cardiomegaly was present in four patients, all of whom were in congestive failure. No patient in our series had a right-sided aortic arch. Characteristic x-rays are shown in Figures 5 and 6.

Table 1 presents the pertinent electrocardiographic findings. Figure 7 analyzes the degree of right ventricular hypertrophy on the basis of the height of the R waves in V_4R or V_1, wherever it was maximal without being in the transitional zone. Nine patients had no significant right ventricular hypertrophy, while thirty-six had maximal R waves of 10 mm. or over. Figure 8 correlates the height of the R waves with the other pertinent electrocardiographic findings. There seems to be no correlation between the degree of right ventricular hypertrophy and the presence of incomplete right bundle branch block whereas there is good correlation between the degree of right ventricular hypertrophy and the presence of p-pulmonale and ST-T wave changes extending from V_1 to at least V_4. Figure 9 shows a typical electrocardiogram.

TABLE 1

ELECTROCARDIOGRAPHIC FINDINGS IN FIFTY PATIENTS WITH ISOLATED PULMONARY STENOSIS

Right ventricular hypertrophy*	41
Right auricular hypertrophy (p-pulmonale)†	19
S-T segment and T wave changes‡	17
Incomplete right bundle branch block§	19

* Criteria defined by Alimurung,[20] Myers[21] and others.

† Peaked P waves of 3.0 mm. or more in lead II and right-sided chest leads.[22]

‡ S-T segment depression of 1 mm. or more and T wave inversion extending from V_1 to at least V_4.[20]

§ Presence of secondary R wave in right-sided chest leads.[23]

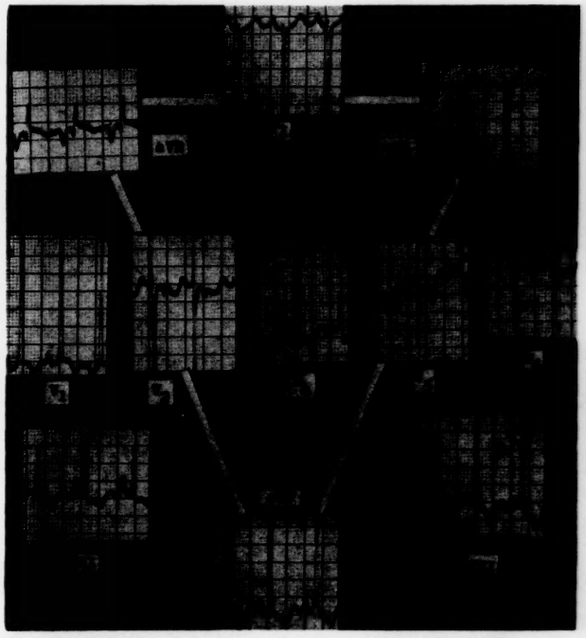

FIG. 9. Typical electrocardiogram of patient with severe pulmonary stenosis.

Physiologic Findings. Right ventricular hypertension[24] was demonstrated in all our catheterized patients. As previously pointed out, in all but two instances the presence of a gradient between the right ventricular and the pulmonary arterial systolic peak pressure was established at catheterization. The abrupt rise in pressure on withdrawing the catheter from the pulmonary artery into the right ventricle suggested the presence of a valvular type of stenosis in forty patients. (Fig. 10.) Thirty-two of these patients had poststenotic dilatation by x-ray. In all surgically treated patients in whom the cardiac catheterization data suggested a valvular stenosis, an obstruction at the valve site was in fact encountered. In two patients no definite preoperative identification of the site of obstruction could be made since the catheter failed to enter the pulmonary artery; at operation both proved to have a valvular type stenosis. In the remaining three patients pressure tracings suggested the presence of an infundibular chamber. (Fig. 11.) The validity of this assumption has not been tested at operation or at autopsy.

The presence of a right-to-left shunt could be assumed with certainty in six patients whose resting arterial oxygen saturation was below 90 per cent. In five others the arterial oxygen saturation was borderline, between 90 and 94 per cent.

A left-to-right shunt at the atrial level, strongly suggesting the presence of an atrial septal defect rather than a patent foramen ovale, was found in three subjects. The calculated shunts were small (under 2.8 L./min.) in all three instances.

Figure 12 demonstrates graphically the wide range of right ventricular peak pressures and the relatively narrow range of pulmonary artery mean pressures. Obviously this suggests that right ventricular pressures are raised to that point which is necessary to maintain a minimal pulmonary arterial mean pressure compatible with life.

Table II demonstrates that patients with definite arterial unsaturation have high right ventricular systolic peak pressures. Of the two patients who had borderline arterial unsaturation with right ventricular pressures in the lower ranges, one certainly had an atrial septal defect as shown by the simultaneous presence of a left-to right shunt. These data strongly suggest that

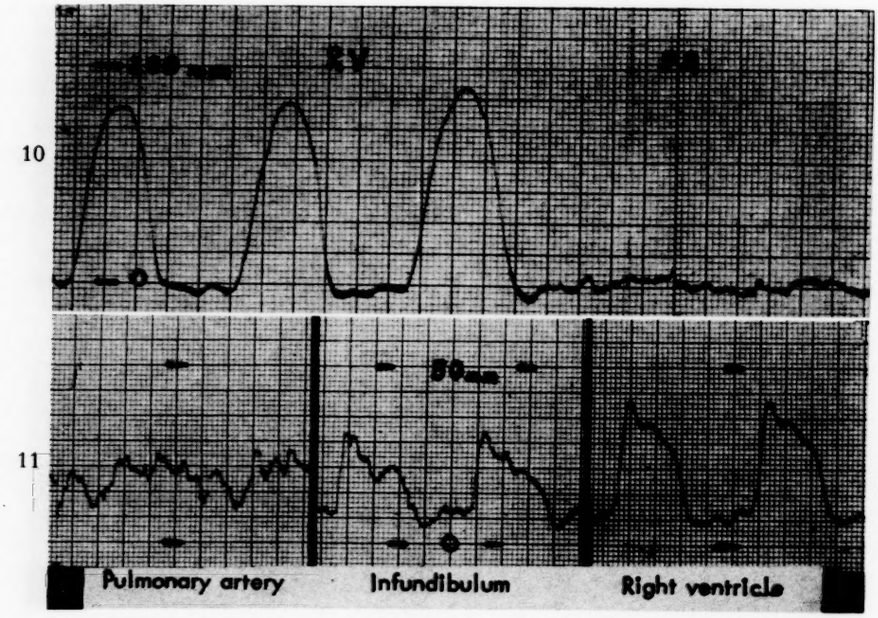

FIG. 10. Typical pressure tracing obtained in a patient with severe valvular stenosis by passing the intracardiac catheter from right ventricle to pulmonary artery.

FIG. 11. Typical pressure tracing in a patient with infundibular chamber demonstrated by withdrawal of catheter from pulmonary artery into right ventricle.

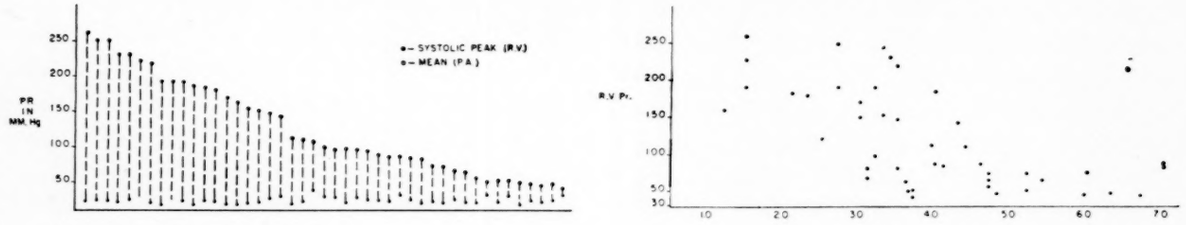

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FIG. 12. Gradient between right ventricular peak and pulmonary arterial mean pressures in forty-five catheterized patients. R.V., right ventricle; P.A., pulmonary artery.

FIG. 13. Relation of resting pulmonary flow index (L./min./M²), abscissa, to right ventricular peak pressure, mm. Hg.

in a patient with isolated pulmonary stenosis the presence of definite cyanosis indicates a rather high degree of obstruction in the right ventricular outflow tract. This observation is not in complete agreement with some data in the literature[11,12] but it is in full accord with Campbell's[18] finding that all his cyanotic patients had marked degrees of right ventricular hypertension.

Analysis of the right auricular pressure tracings revealed the presence of giant "A" waves[25] in fourteen instances. (Table II.) In twelve of these patients the right ventricular pressure exceeded 150 mm. Hg, in the other two it was over 100 mm. Hg.

The cardiac output could be determined only at rest since all but two of these patients were children who could not be exercised adequately during catheterization. Systemic and pulmonary flows were not significantly different from each other because shunts, if present, were of relatively small size. When the height of right ventricular pressure is correlated with the pulmonary flow index (Fig. 13), patients with the higher right ventricular pressures are found to have the lower levels of pulmonary blood flow.

The relationship between calculated valve size[17] and right ventricular peak pressure is presented in Figure 14. As expected, the smallest orifices were associated with the highest pressures.

Clinical and Physiologic Correlations. Since it would be highly desirable, if possible, to separate by clinical means the patients with

severe stenosis from those who have only mild obstruction, we have compared the pertinent clinical data with right ventricular peak systolic pressures. (Fig. 15.) The catheterized patients are arbitrarily divided into two groups—those with right ventricular pressures of 100 mm. Hg or over and those with pressures below this level. It may be seen that the presence of a murmur, the detection of a thrill, the intensity of the pulmonic second sound, and the radiologic evidences of right ventricular enlargement do not help to separate the two groups. On the other hand, patients in the group with high right ventricular pressure were somewhat more likely to have symptoms, although this correlation was far from conclusive. A rather close parallelism was observed between the degree of pulmonary ischemia by x-ray and the right ventricular pressure.

TABLE II
CORRELATION OF RIGHT VENTRICULAR SYSTOLIC PEAK PRESSURES WITH ARTERIAL UNSATURATION AND WITH PRESENCE OF GIANT "A" WAVES *

Right ventricular pressure (mm. Hg):	200–250	150–199	100–149	50–99	under 50
Distribution of patients...	7	8	5	13	12
No. with saturation under 90%	3	3	..	..	..
No. with saturation 90–94%	..	2	1	2	..
No. with giant "A" wave	7	5	2	..	..

* Forty-five catheterized patients (forty-two valvular stenosis, three infundibular stenosis).

The best correlation, however, is found between electrocardiographic findings and right ventricular pressure. All twenty patients who had a right ventricular pressure of 100 mm. Hg or more showed one or several of the following electrocardiographic changes: (1) an R wave in V_4R or V_1 of 20 mm. or over; (2) "p-pulmonale"; (3) abnormal right-sided S-T segment and T wave changes. Conversely, none of the twenty-five patients with right ventricular pressures below 100 mm. Hg showed abnormal S-T segments, T wave changes, or "p-pulmonale"; only one patient in this group had an R wave in V_1 over 20 mm. in height.

Operative Results. Twenty-one patients of the group of fifty were operated upon using the Brock technic.[9] The operative data are presented in Table III. Fifteen of the patients who came to surgery had exercise intolerance of varying severity. Three had congestive failure with marked cardiomegaly. Significant electrocardiographic changes, as previously defined, were observed in all twenty-one. All had right ventricular pressures of 100 mm. Hg or more. Only the three patients in clinical congestive failure died; they were all lost on the operating table.

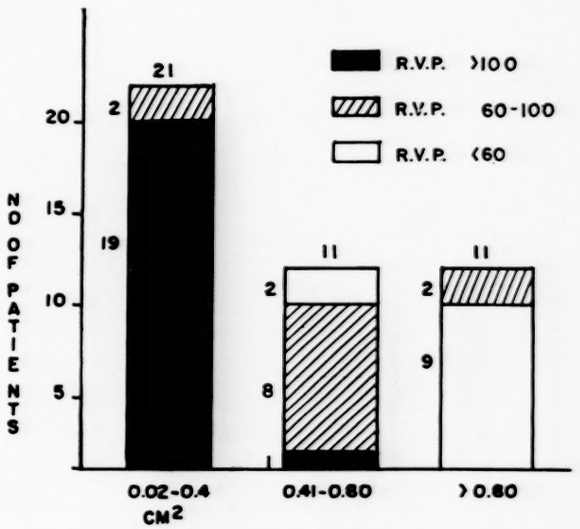

FIG. 14. Calculated valve size in cm.², related to right ventricular peak systolic pressure. R.V.P., right ventricular pressure.

Of the eighteen surviving patients, complete follow-up is not available in ten for a variety of reasons but information obtained by correspondence with the family or with the local physician indicates that symptomatic relief was obtained in all.

Complete postoperative study, including cardiac catheterization, was accomplished in eight patients. The pre- and postoperative findings are compared in Table IV. It may be seen that improvement in exercise tolerance was noted in every instance. Some increase in the pulmonary vascular markings was noted in six. Marked improvement in electrocardiographic features was noted in seven. We have seen significant improvement in the electrocardiogram within a few days after operation, casting doubt on the classic assumption that these changes are solely related to muscular hypertrophy.

Catheterization data demonstrated a postoperative pressure of 86 mm. Hg or less in all but one patient. The pressure was reduced to 40 mm. Hg or less in three. The one child (Case 14) who failed to obtain significant benefit from

TABLE III

PULMONARY STENOSIS WITH INTACT VENTRICULAR SEPTUM—OPERATIVE DATA IN TWENTY-ONE PATIENTS

Case No. and Patient	Age*	Date of Operation	Exercise Intolerance	Congestive Heart Failure	Gross Cardiomegaly	Significant EKG Changes	Right Ventricular Systolic Pressure (mm. Hg)	Result
1, M. Z.	5 yr., 6 mo.	7/51	+	0	0	+	240	Good
2, E. G.	11 yr., 10 mo.	1/52	+	+	+	+	219	Died
3, C. H.	10 yr., 10 mo.	5/52	+	0	0	+	250	Good
4, D. C.	4 yr., 10 mo.	6/52	+	0	0	+	220	Good
5, W. U.	8 yr., 4 mo.	7/52	+	0	0	+	180	Good
6, J. M.	8 yr., 1 mo.	7/52	+	0	0	+	170	Good
7, A S.	10 yr.	7/52	+	0	0	+	140	Good
8, D. G.	8 yr., 4 mo.	12/52	+	0	0	+	134	Good
9, T. C.	6 yr., 11 mo.	1/53	+	0	0	+	. . .	Good
10, P. M.	4 yr., 11 mo.	1/53	+	0	0	+	. . .	Good
11, P. F.	12 yr., 4 mo.	5/53	+	0	0	+	160	Good
12, W. C.	5 mo.	10/53	. .	+	+	+	150	Died
13, M. R.	21 yr., 5 mo.	11/53	+	0	0	+	102	Good
14, E. P.	9 yr., 3 mo.	11/53	+	0	0	+	180	Fair
15, J. C.	21 yr.	3/54	+	0	0	+	180	Good
16, W. V.	5 yr.	3/54	0	0	0	+	210	Good
17, G. E.	14 yr.	5/54	+	+	+	+	136	Died
18, H. D.	6 yr.	6/54	0	0	0	+	208	Good
19, M. M.	11 yr., 9 mo.	7/54	0	0	0	+	174	Good
20, C. S.	5 yr., 1 mo.	8/54	0	0	0	+	175	Good
21, J. T.	4 yr., 10 mo.	8/54	0	0	0	+	144	Good

* At time of operation.

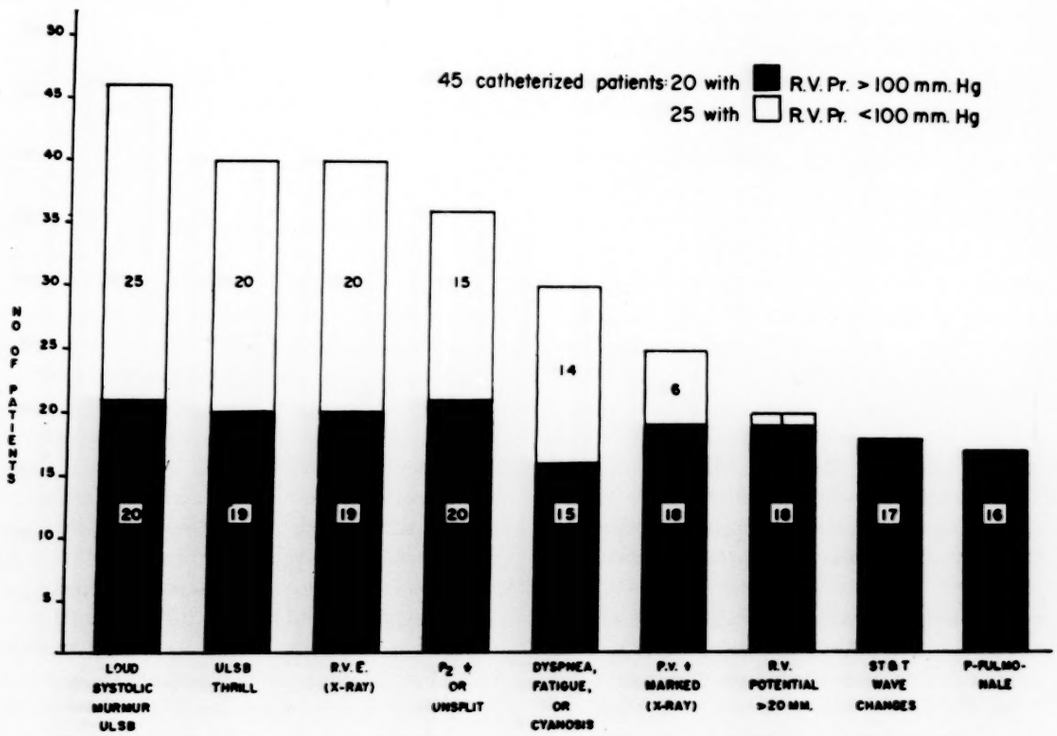

FIG. 15. Clinical data related to right ventricular systolic pressure. Abbreviations as before.

TABLE IV

PRE- AND POSTOPERATIVE DATA IN EIGHT PATIENTS

Case No. and Patient	Months after Operation	Exercise Intolerance		Pulmonary Vasculature*		Electrocardiogram						Catherization	
						Height R Wave in V_1 (mm.)		Auricular Hypertrophy		ST-T Wave Changes		Right Ventricular Peak Systolic Pressure (mm. Hg)	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1, M. Z.	18	+	0	Dim.	Low N	32	10	+	0	+	0	240	30
4, D. C.	22	+	0	Dim.	Less dim.	39	20	+	0	+	0	220	86
5, W. U.	24	+	+	Dim.	Low N	24	15	+	0	+	0	180	68
6, J. M.	20	+	0	Dim.	N	40	6	+	0	+	0	170	36
7, A. S.	11	+	0	Dim.	Low N	14	4	+	0	0	0	140	41
9 T. C.	18	+	+	Dim.	Dim.	36	25	0	0	0	0	. . .	70
10, P. M.	18	+	0	Dim.	Less dim.	30	15	0	0	0	0	. . .	80
14, E. B.	6	+	0	Dim.	Dim.	31	30	+	+	+	+	180	50

* Dim. = diminished; N = normal.

operation, in physiologic terms, has nevertheless experienced complete relief of preoperative symptoms. It should be noted also that this child was the only one who failed to show improvement in his electrocardiogram.

Figure 16 gives a graphic demonstration of the postoperative changes in right ventricular pressure and in right ventricular potentials in the electrocardiogram. It may be seen that there is a close parallelism between the drop in right ventricular pressure and the diminution of right ventricular potentials noted postoperatively. These data, together with the correlations presented in Figure 15, clearly point to the electrocardiogram as a sensitive indicator of the height of the right ventricular systolic pressure.

Because of the low operative mortality figures and the good clinical and physiologic improvement which has been obtained in the majority of cases, we do not feel justified in abandoning the rather simple and satisfactory Brock technic for the recently proposed[14] open valvulotomy employing hypothermia.

THERAPEUTIC CLASSIFICATION AND MANAGEMENT

According to the data presented, it is possible to classify our patients with "pure" pulmonary stenosis into three groups.

The *first* group is exemplified by the four subjects who died in infancy, representing a most serious variety of the disease.[15,26] In only two of these was the diagnosis made before death and in only one was there sufficient time to attempt detailed study and operation. These infants are recognizable by the characteristic clinical signs of severe "isolated" pulmonary stenosis, accompanied by evidences of right-sided congestive failure, i.e., a large heart, distended neck veins, hepatomegaly and possibly peripheral edema. Physiologic data obtained in one of these patients (Case 12) showed a right ventricular pressure of 150 mm. Hg and a pulmonary flow index of 1.3 L./min./M^2. Autopsy findings in all of these patients showed maximal stenosis of the pulmonary valve, with only a pinhole orifice. We believe that in this group operation should be attempted as soon as the diagnosis is made. Anticongestive measures (digitalis and mercurial diuretics) should be taken preoperatively, although their effectiveness in our experience has not been impressive.

A *second* group is represented by patients who have classic findings of "pure" pulmonary stenosis with clinical indications of a right ventricular pressure of 100 mm. Hg or over. (Fig. 15.) All these cases are recognizable by the

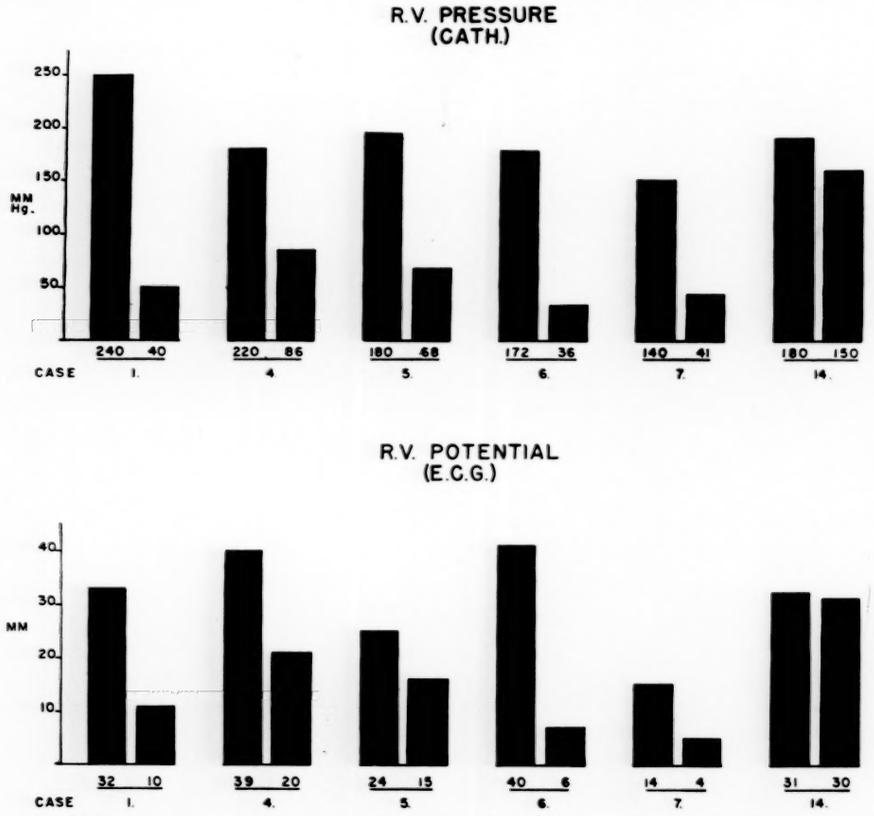

FIG. 16. Pre- and postoperative data in six patients. Left hand column, preoperative figure; right hand column, postoperative figure.

significant electrocardiographic changes already defined. Definite clinical symptomatology and marked diminution of the pulmonary vasculature by roentgenogram was present in the majority of patients in this group.

The patients in this category deserve cardiac catheterization to ascertain the right ventricular pressure. This group of patients will benefit from valvulotomy and probably all should be operated on at some time. The exact timing of the operation will depend on the severity and progression of the findings, particularly symptomatology, electrocardiograms and x-rays. It is our belief that patients with right ventricular pressure over 100 mm. Hg and significant symptoms should be operated upon promptly. There is little experience at this time, however, to indicate when patients with right ventricular pressures of 100 mm. Hg or more but without symptoms should be operated upon. The authors believe that there is indication for surgical intervention in this group if one or more of the following conditions exists: (1) significant cardiomegaly; (2) electrocardiographic finding of progressive increase in the height of the R wave in right-sided chest leads; (3) electrocardiographic finding of ST-T wave changes.[27] If operation in this group is temporarily postponed, adequate follow-up at three- to six-month intervals is emphatically recommended.

The *third* group is comprised of patients whose x-rays and electrocardiograms suggest right ventricular pressures below 100 mm. Hg. (Fig. 13.) These patients may or may not have symptoms; their electrocardiogram will, however, show right-sided R waves of less than 20 mm. without p-pulmonale and with no significant ST or T wave changes; their x-rays are more likely to show normal or only slightly diminished pulmonary vasculature.

At the present time we do not believe that it is necessary to catheterize these patients. Instead, we follow them at six-month to one-year intervals and observe them for possible findings which would indicate that their right ventricular pressures are now in the higher group. We do not operate upon these patients, at this time, regardless of symptomatology. We believe that

many of these children have a mild form of the disease that may never require correction. The data in the literature indicate that operation using the Brock technic usually does not result in a satisfactory pressure drop in this group.[11,12] It is even suggested that surgery may damage the functional adequacy of the valve.[28] We believe that careful follow-up at the intervals mentioned will allow adequate time to observe any change in their status.

SUMMARY

1. Data on fifty patients with proved pulmonary stenosis and intact ventricular septum are presented.

2. The symptomatology is dominated by severe dyspnea and fatigue, and mild if any cyanosis. About 25 per cent of the patients have no symptoms.

3. The characteristic findings include a rough systolic murmur with a diminished second sound at the upper left sternal border, evidence of right ventricular hypertrophy in the electrocardiogram, and right ventricular enlargement, prominent main pulmonary artery and diminished pulmonary vasculature in roentgenograms.

4. Physiologic data reveal varying degrees of right ventricular hypertension and a systolic gradient across the pulmonary valve. The pressure tracings suggest the presence of a valvular stenosis in the majority of instances. Arterial unsaturation at rest always indicates severe stenosis and is found in about 10 per cent of the cases.

5. A significant correlation between the electrocardiographic findings of right ventricular hypertrophy and right ventricular systolic pressure could be established in this series.

6. Operative results in twenty-one patients subjected to Brock valvotomy are presented. There were three fatalities, all in patients exhibiting failure. All the survivors showed clinical improvement. In seven of eight patients studied by cardiac catheterization postoperatively, a satisfactory drop in right ventricular pressure was achieved.

7. A therapeutic classification is proposed: (*a*) Infants with severe stenosis in congestive failure should be operated on as soon as the diagnosis is made. (*b*) Patients with clinical findings suggesting right ventricular pressures of 100 mm. Hg or over usually should be operated upon at an appropriate time. (*c*) Patients in whom findings indicate that the right ventricular pressure is probably under 100 mm. Hg should be observed carefully but need not be subjected to cardiac catheterization or to operation.

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Atrial Flutter with 1:1 A-V Conduction*

Report of Six Cases

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ATRIAL flutter with varying degrees of A-V heart block is not an uncommon arrhythmia but its presence with 1:1 conduction is rare and constitutes a serious cardiac emergency. The rapid ventricular rate associated with 1:1 flutter results in marked subjective distress, accompanied usually by symptoms and signs of cardiac failure with varying degrees of shock. If tachycardia continues, progressive cardiovascular deterioration and death result within a relatively short period.

The first documented case of 1:1 atrial flutter was reported by Lewis[1] in 1915. Since then about forty additional cases have been described in the literature.[2–17,19–22,24–27,29,31] We have studied six such cases, two of whom had repeated bouts of 1:1 flutter.

Clinically, the presence of this arrhythmia was suspected because of sudden onset of a rapid, regular ventricular rate which ranged from 225 to 315 per minute. Failure to respond to carotid sinus pressure was noted in all but one patient who had been receiving digitalis. The diagnosis of 1:1 flutter was established in our patients by means of the electrocardiogram taken before, during and after therapy. Diagnosis by electrocardiogram is often difficult to make with certainty because rapid ventricular complexes often mask the characteristic atrial waves. The electrocardiographic criteria considered by us to be suggestive of 1:1 flutter were as follows: (1) the presence of an atrial wave (rate 225 to 315 per minute) that consisted of an undulating or oscillating motion with a saw-tooth appearance which never displayed any isoelectric period, accompanied by a ventricular response to each atrial beat; (2) demonstration of atrial flutter with higher degrees of A-V block (2:1, 3:1, and the like) either before or after onset of 1:1 arrhythmia is presumptive evidence that the latter is due to atrial flutter; (3) the response to treatment of an ectopic rhythm with a rapid ventricular rate helps confirm the diagnosis: following digitalis therapy, the appearance of atrial flutter with increasing degrees of A-V block (2:1, 3:1, 4:1) or atrial fibrillation lends support to the original provisional diagnosis of 1:1 atrial flutter.

CASE REPORTS

CASE 1. D. W., a sixteen year old Negro woman, was well until August 28, 1947, when she suddenly became short of breath and was conscious of a rapid pounding of the heart as she walked up the front steps of her house; this was soon followed by a fainting sensation. This triad of symptoms persisted for the next twenty-four hours when she was admitted to the Chester Hospital. The patient had suffered a similar episode of tachycardia one month prior to the present attack but it had subsided spontaneously within one hour. The past medical history was non-informative.

Examination revealed a well developed young woman in no great distress; no signs of recent infection were present. The thyroid gland was of normal size. No evidence of congestive failure was noted and the heart appeared normal in size; there was a regular rhythm with a ventricular rate of 300 per minute. No murmurs were audible and the blood pressure was 85/50. Orbital pressure and repeated carotid sinus pressure on both sides were ineffective. An electrocardiogram was interpreted as showing atrial flutter with 1:1 conduction (rate of 315 per minute), widened QRS complexes and right axis deviation.

The patient was given quinidine sulfate by mouth and within eight and one-half hours she had received a total of 3.4 gm. At this time an electrocardiogram revealed persistence of the flutter with 1:1 conduction and a rate of 210 per minute. Therapy was then changed because of toxic manifestations and within

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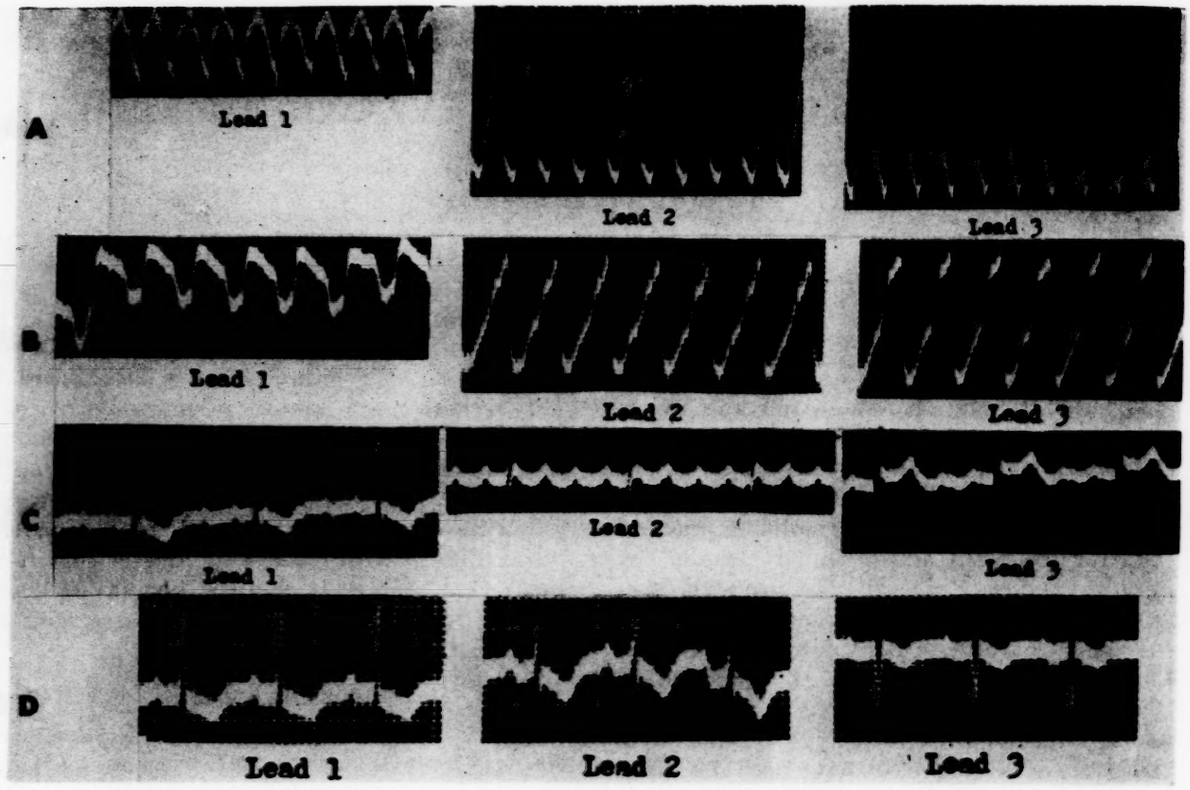

FIG. 1. Case 1. A, August 2, 1948, upon the patient's admission; rate 275 per minute. The atrial mechanism cannot be clearly determined because of the high ventricular rate. QRS complexes are notched and widened. B, August 6, 1948; rate between 187 and 214 per minute, after patient had received digitalis and quinidine medication. The QRS complexes exhibit increased widening as compared to A. C, August 6, 1948; atrial flutter with 4:1 A-V conduction can be seen. The atrial rate is 300 per minute, the ventricular rate 75 per minute. Note the change in axis deviation with return of the QRS complex to normal duration and configuration. D, August 7, 1948, normal sinus rhythm with a rate of 125 per minute and digitalis effects are seen.

the next forty-eight hours 2.7 mg. of digitoxin were given orally without any effect on the arrhythmia; the rate on Sept. 1 was 255 per minute.

The patient's condition deteriorated; vomiting, restlessness and fever (102°F.) developed. The rate on September 2 was 275 per minute and signs of right-sided failure appeared. On this day, therapy consisted of two intramuscular injections of 0.2 mg. each of digitoxin given four hours apart and 0.66 gm. of quinine dihydrochloride dissolved in 250 cc. of physiologic saline solution administered intravenously over a three-hour period. The apical rate dropped to 180 per minute but resumed its former level of 275 per minute within a few hours. A second intravenous dose of 0.66 gm. of quinine dihydrochloride was then given; inadvertently, the solution ran in rapidly (twenty minutes) and the patient complained of blurred vision, became rigid and vomited profusely. The heart rate, still regular, dropped to 102 per minute and then settled down to 90 per minute with marked improvement in the patient's condition. An electrocardiogram taken on September 3 showed normal sinus rhythm, rate 85 per minute, disappearance of the right axis deviation previously noted and evidence of digitalis effect.

Thereafter, repeated cardiac examinations failed to reveal any clinical abnormalities. On September 22, 1947, the patient was discharged from the hospital feeling well.

Electrocardiograms taken on this admission were identical with those of the second admission, shown in Figure 1.

Second admission: After leaving the hospital the patient took no medication and felt well until August 1, 1948 when she became excited while running for a bus and noted sudden onset of a rapid heartbeat accompanied by dyspnea. Because of the persistence of these symptoms she was brought back to the Chester Hospital at 11:30 P.M. when the apical rate was recorded as 300 per minute. No evidence of congestive failure was found. She was given 0.6 gm. of quinidine sulfate by mouth every three hours for four doses without any effect on the tachycardia. An electrocardiogram taken on the morning following admission was interpreted as showing atrial flutter with 1:1 conduction and a rate of 275 per minute. (Fig. 1A.)

The tachycardia continued notwithstanding ingestion of an additional 1.2 gm. of quinidine and intravenous injection of 0.66 gm. of quinine hydrobromide given by the drop method over a period of two hours. In the next forty-eight hours the patient received 2.1 mg. of digitoxin intramuscularly and 1.4 mg. orally in divided doses without any change in the arrhythmia.

The patient was febrile (102–103°F.), dyspneic and restless; considerable vomiting and prostration were present but no signs of congestive failure were noted. Penicillin was ordered and on August 4 (third hospital day) she received 1.0 gm. of procaine hydrochloride intravenously, three two hourly doses of 0.5 mg. each of prostigmin® and one cat unit of digalen® subcutaneously, without effect. A second dose of 1.3 gm. of quinine hydrobromide and 0.25 mg. of prostigmin diluted in 200 cc. of water was then given intravenously without effect. This was followed by intravenous injection of 1.0 mg. of ouabain given in three divided doses at two hour intervals, without any demonstrable change in the rate or rhythm.

The patient's condition deteriorated notwithstanding supportive treatment. On August 5, 0.65 gm. of quinidine sulfate diluted in 100 cc. of water was given intravenously every two hours for three doses, followed by 450 cc. of 1.14 per cent potassium chloride solution; this resulted in temporary slowing of the ventricular rate to 160 per minute. On August 6 the patient's condition became worse; the blood urea nitrogen was reported to be 79 mg. per cent, the creatinine was 2.5 mg. per cent and the CO_2 combining power was 30 vol. per cent. In addition to intravenous fluids, she received 15 mg. of mecholyl® intramuscularly without effect on the heart rate; the electrocardiogram showed widening of the QRS complexes and 1:1 flutter with a rate of 195 per minute. (Fig. 1B.)

At 7:30 P.M. the patient showed intense exhaustion and cyanosis, and 0.25 mg. of prostigmin was given intravenously, followed in fifteen minutes by 0.5 mg. of ouabain. At 8:00 P.M. the cardiac rate was 240 per minute. At 8:30 P.M. the apical rate dropped to about 88 per minute and was irregular. The blood pressure, which had been unobtainable during the day rose to 125/75. At 10:30 P.M. the heart rate was 80 per minute and 0.2 mg. of ouabain was then given intravenously; the electrocardiogram at this time showed atrial flutter with 4:1 A-V block and a ventricular rate of 75 per minute. A marked S-T segment deviation was observed, probably the result of digitalis therapy. (Fig. 1C.)

The following morning (August 7) the apical rate was 70 per minute and regular; an electrocardiogram showed the presence of sinus rhythm. (Fig. 1D.) Blood pressure was 125/75. The patient appeared drowsy but responded to simple orders. No localizing neurologic signs were found with the exception of some nuchal resistance to flexion. Spinal fluid examination failed to reveal any abnormalities. Portable x-ray examination of the chest gave negative results. The blood urea nitrogen was 57 mg. per cent and the CO_2 combining power was 55 vol. per cent. The white blood count was 13,200 with 87 per cent polymorphonuclear cells. Notwithstanding continued supportive treatment, the patient sank into deep coma and died on August 9, 1948 at 8:10 A.M.

The following pertinent observations were made at autopsy:* the heart was normal in size and appearance with the exception of a small subendocardial hemorrhage and the presence of petechiae. Passive congestion of the viscera, terminal confluent bronchopneumonia and a small renal infarct were noted. Microscopic examination revealed the presence of patchy subendocardial and epicardial fibrosis with areas of calcification, calcium deposits in the renal tubules, passive congestion of the lungs, liver, spleen and kidneys, and a small colloid nodule of the thyroid. The cause of death was not established by autopsy.

CASE II. B. K., a thirty year old married white woman was admitted to the Crozer Hospital, Chester Pennsylvania, March 4, 1950. She had been well until the afternoon of admission when, while shopping, she suddenly became conscious of a rapid heart beat, tremor within the chest, a fainting sensation and blurring of vision. Examination by her family physician revealed the heart action to be regular, "around 300 per minute," and unaffected by carotid sinus pressure. The patient was given a hypodermic injection of morphine sulfate (¼ gr.) and admitted to the hospital about one and one-half hours after onset of tachycardia. No history of previous heart disease, rheumatic fever, thyroid disorder or recent infection was noted. The patient had had a great deal of financial worry and domestic tension. Physical examination revealed a young woman in great distress because of throbbing in the head and a fainting sensation. Dyspnea was not present. The lungs were clear and the heart was regular with a rate of 250 per minute. Blood pressure was not obtainable. Carotid sinus pressure was ineffective. The electrocardiogram was reported as showing atrial flutter with 1:1 conduction. (Fig. 2A.) Ouabain, 0.6 mg., was given intravenously and within three minutes the heart rate slowed and became totally irregular with a ventricular rate of 132 per minute. (Figure 2B.) This was followed by an intramuscular injection of three cat units of digalen. Four hours later, an additional cat unit was administered.

On the following morning the patient was much improved. The heart appeared to be normal except for the presence of coupled ventricular extrasystoles (Fig. 2C) and the blood pressure was 110/70. Routine laboratory tests, basal metabolic rate and x-ray of the chest were all within normal limits. She was discharged, feeling well, on March 7, 1950. A follow-up examination on March 29, 1950 failed to reveal any

* Performed by Dr. George Sickel.

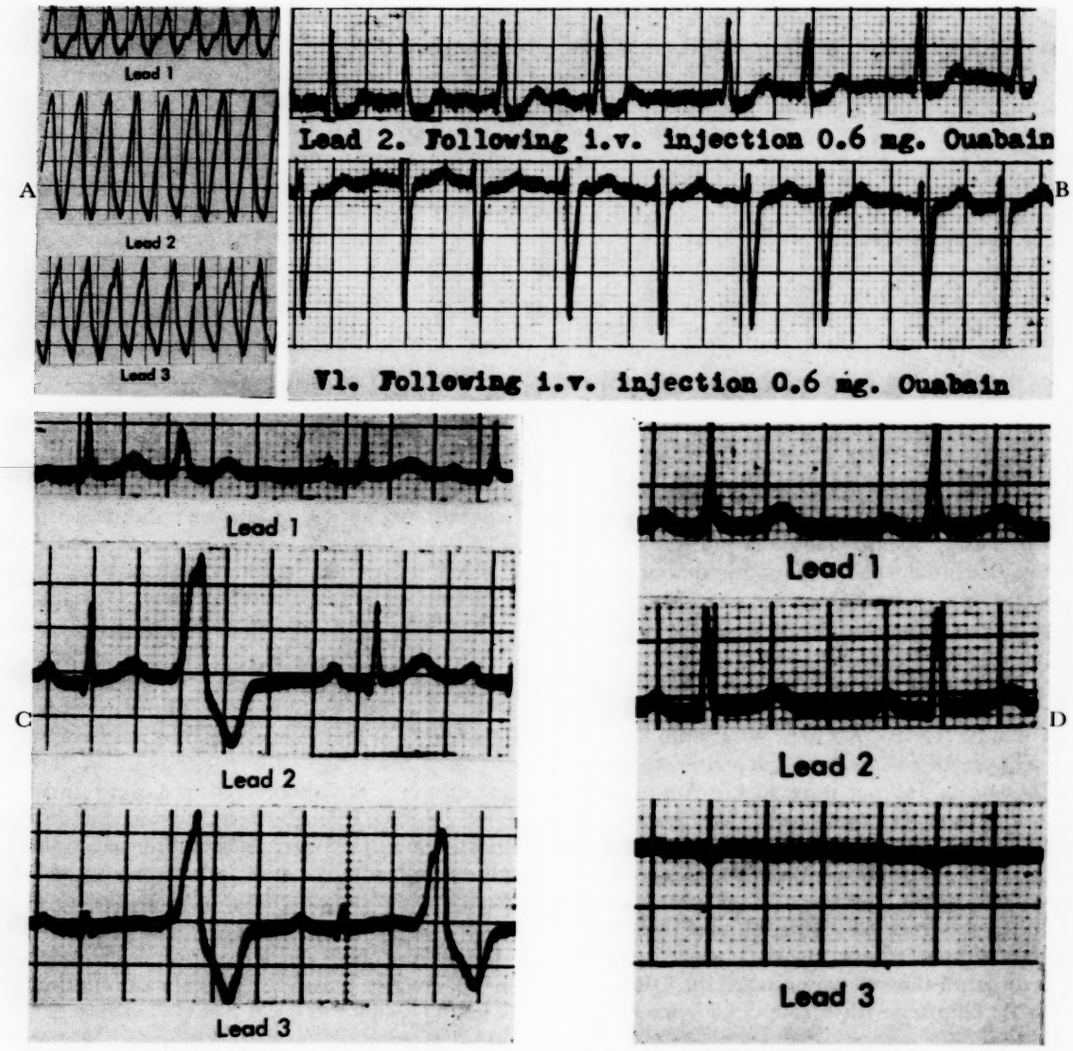

FIG. 2. Case II. A, March 4, 1950; note notched and widened QRS complexes with a rate of 250 per minute. B, ouabain (intravenous injection, 0.6 mg.) changed the atrial mechanism to fibrillation and the ventricular rate dropped to 120 to 132 per minute. The QRS complex then displayed normal width. This sequence of events suggests that the rhythm in A was due to atrial flutter with 1:1 response. C, March 5, 1950; normal sinus rhythm has been restored. Coupled ventricular extrasystoles are present, the result of digitalis overdosage. D, March 29, 1950; patient was asymptomatic and taking no medication. The P-R interval is at the upper limit of normal; the tracing is otherwise within normal limits.

cardiac abnormality. The orthodiagram and electrocardiogram were normal. (Fig. 2D.)

CASE III. J. P., a forty-six year old married white man, was admitted to the Chester Hospital on April 13, 1951 with a presumptive diagnosis of myocardial infarction. The patient had been well and working when, after heavy exertion, he suddenly developed a rapid heart beat, precordial pain, dyspnea and profuse sweating. He had had an attack of rheumatism in 1935 and shortly thereafter had been told of the existence of a murmur. During a routine examination in February, his physician found the heart rate to be irregular; an electrocardiogram taken on March 15 showed atrial fibrillation with a ventricular rate of 110 per minute and evidence of left ventricular hypertrophy.

Upon admission to the hospital the apical rate was regular at 250 per minute, the heart sounds were faint, the blood pressure was unobtainable and fine rales were heard at both bases. An electrocardiogram was interpreted as showing atrial flutter with 1:1 conduction and a rate of 250 per minute. (Fig. 3A.) Carotid sinus pressure was ineffective.

Ouabain, 0.5 mg., was injected intravenously at 4:30 P.M. and gave no immediate results. Digitalization was effected by means of five cat units of digalen

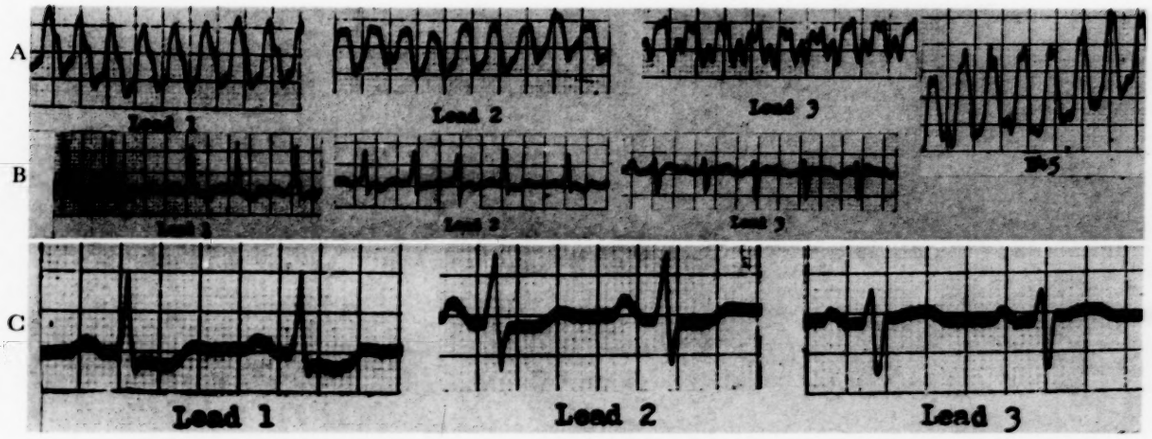

FIG. 3. Case III. A, April 13, 1951, upon admission, regular tachycardia, rate 250 per minute. Note bizarre, aberrant QRS complex which shows considerable widening. Esophageal lead at 45 cm. shows an intrinsicoid P wave preceding each ventricular complex, suggesting that a 1:1 atrial flutter may be the cause of the tachycardia. B, five hours after intravenous injection of ouabain, 0.5 mg.; atrial rhythm has changed to fibrillation and ventricular rate is now 135 per minute. The QRS complex now shows normal width. C, April 20, 1951, after normal sinus rhythm was restored by means of quinidine. Evidence of quinidine effects is suggested by slightly delayed intraventricular conduction of the QRS complex and prolonged QT interval (0.44 seconds). The P wave is wide and slurred, probably the result of rheumatic mitral heart disease.

injected intramuscularly; 2.0 cc. of thiomerin and morphine sulfate (¼ gr.) were given hypodermically. This medication was followed by two bouts of vomiting. At 9:00 P.M. the apical rate had dropped to 130 per minute and was irregular; an electrocardiogram (Fig. 3B) revealed the presence of atrial fibrillation with an average ventricular rate of 135 per minute. The patient appeared to be much improved. The heart was enlarged to the left. A faint systolic aortic murmur with an aortic second sound of diminished intensity and a loud harsh systolic mitral murmur were heard, but no diastolic rumble was demonstrable. The blood pressure was 140/88.

During the first two days of hospitalization the patient displayed atrial fibrillation with a well controlled ventricular rate. The signs of pulmonary congestion had cleared and the blood pressure was 140/80. X-ray examination of the heart showed the presence of left ventricular and left auricular enlargement. On April 16 the patient was given quinidine sulfate, 0.4 gm. every four hours, and after two days sinus rhythm was restored and maintained. (Fig. 3C.) He was discharged on April 21, 1951, improved and fully compensated.

CASE IV. H. P., a sixty year old white man, was admitted to the Graduate Hospital on November 21, 1951 for frontal lobotomy because of intractable back pain due to metastatic carcinoma of the prostate. Past history revealed several hospital admissions, one on March 21, 1948 for a bleeding peptic ulcer, another on May 31, 1949 for a suprapubic prostatectomy for carcinoma of the gland. At this time an electrocardiogram revealed the presence of atrial flutter with varying ventricular response (2:1, 3:1 or 4:1). This arrhythmia was again noted on June 5, 1950 when the patient underwent a bilateral subcapsular orchiectomy. During this interval digitalis therapy was maintained.

At the time of the present admission (November 21, 1951) the patient had no complaints relative to the heart. Examination revealed the following significant findings: cachexia, moderate cardiac enlargement to the left, short systolic murmurs over the apex and base of the heart, a regular cardiac rate of 160 per minute and blood pressure of 110/70; no evidence of congestive failure was noted. Metastases to the back and rectum were found. Electrocardiogram revealed the presence of atrial flutter with 2:1 A-V heart block.

Operation was scheduled for November 28, 1951. A preoperative electrocardiogram revealed atrial flutter with an A-V block varying between 3:1 and 4:1. (Fig. 4A.) Prior to the time scheduled for the operation the patient received 0.2 gm. of nembutal® and 30 mg. of codeine sulfate hypodermically. During the induction of anesthesia, which consisted of 10.0 cc. of pentothal® intravenously, 5 per cent cocaine spray to the pharynx for tracheal intubation, and nitrous oxide and oxygen mixture inhalation, a heart rate of 286 per minute developed and the patient went into shock. No blood pressure was obtainable. Electrocardiogram showed the presence of atrial flutter with 1:1 A-V conduction. (Fig. 4B.) Carotid sinus massage on the right side resulted in a 2:1 A-V block (Fig. 4C) with return of the blood pressure to 110/80. Operation was postponed. Further digitalis therapy resulted in higher grades of A-V block. (Fig. 4D.) A successful lobotomy was performed on December 13, 1951, and no further disturbance in the cardiac mechanism was

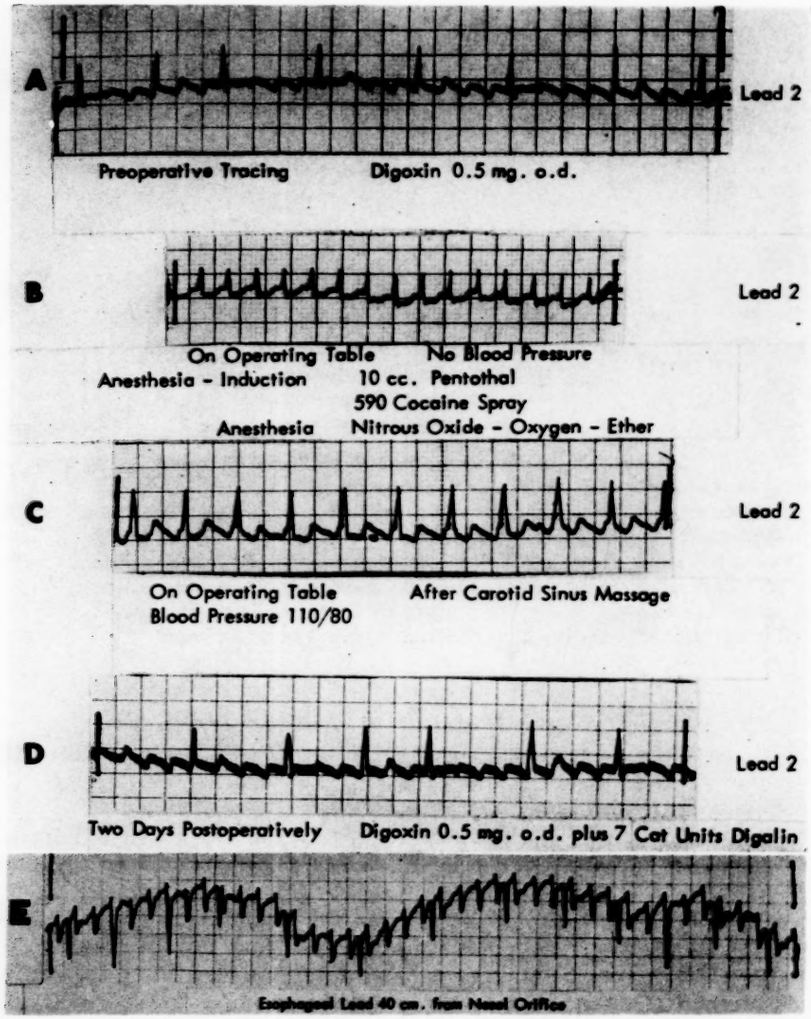

FIG. 4. Case IV. A, atrial flutter with varying degrees of A-V heart block (2:1 to 4:1) is observed in the preoperative tracing. The ventricular rate averages 90 per minute. B, note the presence of 1:1 atrial flutter with a rate of 286 per minute during induction of anesthesia. C, carotid sinus massage resulted immediately in 2:1 A-V heart block. D, further digitalization resulted in higher and varying degrees of a A-V heart block (2:1 to 4:1.) Average ventricular rate is 90 per minute. E, esophageal lead (E 40) shows intrinsicoid P wave with varying degrees of A-V heart block (2:1 to 4:1).

noted. The back pain was relieved and the patient was discharged on December 10, 1951. The laboratory findings were not significant.

CASE V. L. D., a fifty-seven year old white woman, was admitted to the Philadelphia General Hospital on September 4, 1952. She had been well until 1950 when she experienced occasional short paroxysms of rapid beating of the heart, dyspnea on exertion, nervousness and fainting spells. Prior to the hospital admission she had been troubled with abdominal distention, constipation, anorexia and weight loss. On the day of admission the patient was seized with a very rapid beating of the heart accompanied by nervousness, weakness and orthopnea.

Physical examination revealed a semi-stuporous, cold, clammy, cyanotic female in acute distress. Respirations were rapid and shallow, the pulse was 248 per minute, regular and thready, and the blood pressure was unobtainable. The neck veins were distended and the heart was enlarged to the left, but no murmurs were heard. A few rales were audible at the lung bases.

The electrocardiogram revealed an atrial flutter with a rate of 248 per minute with a 1:1 response. (Fig. 5A.) Demerol® (100 mg.), prostigmin (0.5 mg.) and carotid sinus pressure failed to influence the arrhythmia. The intravenous injection of 0.8 mg. of cedilanid® produced a 2:1 A-V block almost im-

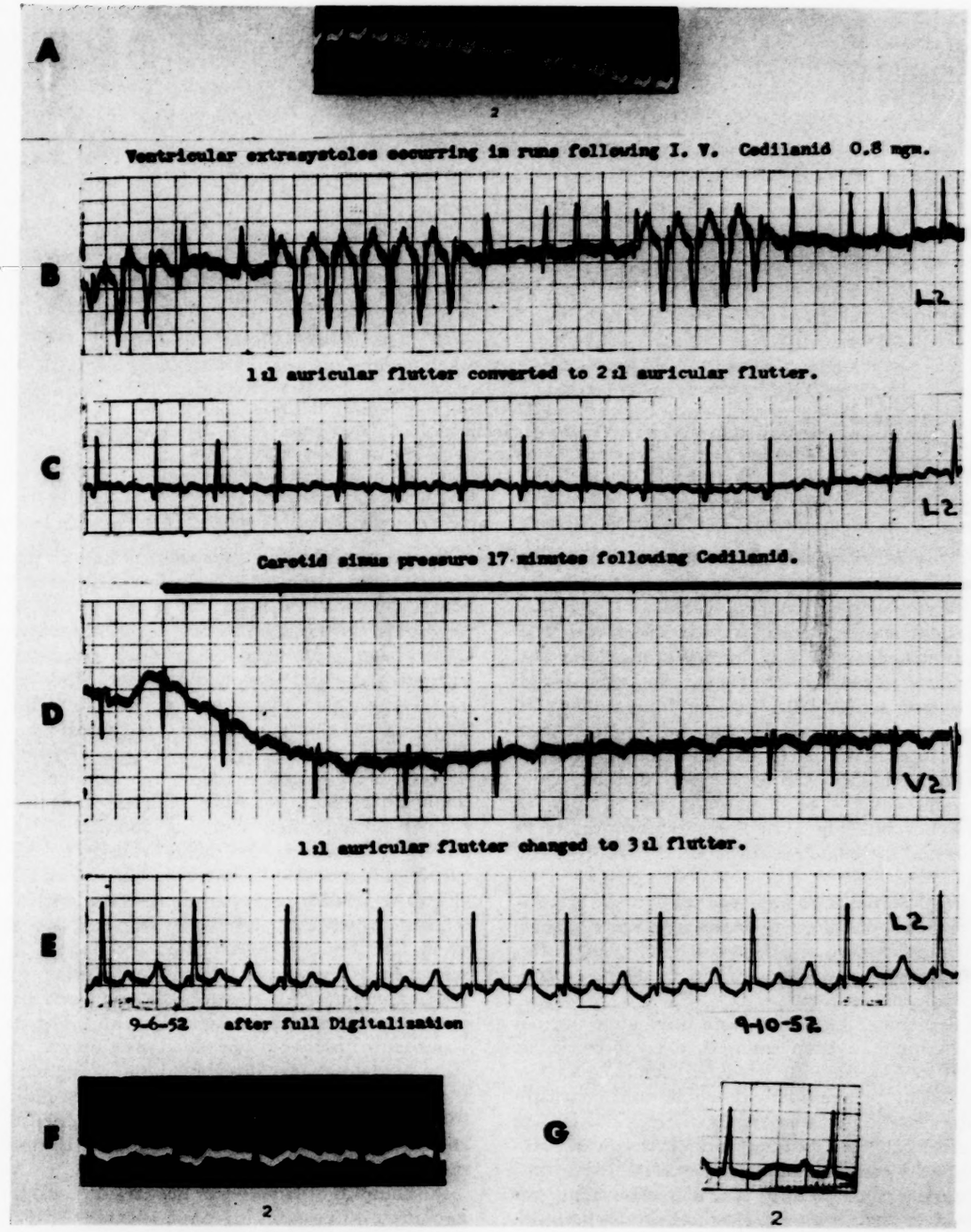

FIG. 5. Case V. A, control tracing (lead II); note rapid regular tachycardia rate, 248 per minute (September 4, 1952). B, intravenous injection of 0.8 mg. of cedilanid resulted in an occasional cycle of atrial flutter with 2:1 A-V heart block and short paroxysms of ventricular tachycardia. C, fifteen minutes after B was taken, 2:1 atrial flutter was recorded. D, carotid sinus pressure resulted in higher degrees of A-V heart block. E, later that day, 3:1 atrial flutter was recorded. F, (September 6, 1952) lead II; after full digitalization, the atrial mechanism was changed from atrial flutter to auricular fibrillation. G, September 10, 1952, lead II; cardiac mechanism has now changed to normal sinus rhythm.

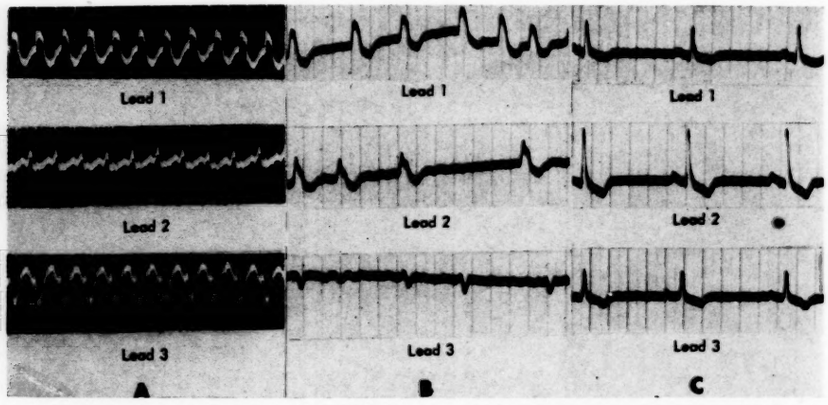

FIG. 6. Case VI. A, January 18, 1952; note rapid regular tachycardia with widened QRS complexes with a rate of 225 per minute. B, January 19, 1952, after 25 mg. of cedilanid intravenously. The atrial mechanism had now changed to atrial fibrillation with an average ventricular rate of 100 per minute. C, January 21, 1952; normal sinus rhythm is now present. The duration of the QRS complexes is now normal. Inverted T waves are probably the result of digitalis effects.

mediately (ventricular rate 120 per minute) which was interrupted during the first forty-five seconds by short runs of ventricular tachycardia. (Fig. 5B.) The patient showed much improvement with the slower ventricular rate and became more alert. The dyspnea and cyanosis diminished and the blood pressure rose to 120/80. With additional digitalis therapy, a 3:1 A-V block developed. (Fig. 5E.) After full digitalization the mechanism changed to auricular fibrillation. (Fig. 5F.) On September 10, 1952, while on a maintenance dose of digitalis, the rhythm reverted to normal. (Fig. 5G.) The patient continued to improve and the blood pressure leveled off at 180/100 on September 8, 1952.

The clinical diagnosis was hypertensive and arteriosclerotic heart disease, left ventricular enlargement; normal sinus rhythm and a paroxysm of atrial flutter with 1:1 response was noted. No evidence of congestive failure was noted.

On September 16, 1952, nine days after normal sinus rhythm had been restored, a cerebrovascular accident developed with left-sided hemiplegia. The patient went progressively downhill and, notwithstanding adequate supportive therapy, died on September 28, 1952. Normal sinus rhythm was maintained to the end. Necropsy was not obtained.

CASE VI. W. H., a fifty year old white man, was admitted to the Graduate Hospital on January 18, 1952 with a history of repeated attacks of rapid beating of the heart since 1928. These attacks were infrequent at first but recently had recurred at intervals of ten to fourteen days. The patient had had over twenty hospital admissions at various institutions for this tachycardia and had received either digitalis, quinidine or procaine amide therapy. The attacks had lasted from a few hours to two days; there had been no chest pain or ankle edema.

His present admission was ushered in with palpitation, dypsnea, cyanosis and obvious distress of seven hour's duration. A history of a twenty-two pound weight loss during the previous six months was noted. Observation in the hospital revealed exophthalmos, widened palpebral fissure, diminished blink and lid lag. The thyroid gland was normal to palpation. The heart was normal in size and the rate was 225 per minute and regular; no evidence of congestive heart failure was present and no murmurs were heard; the blood pressure was 90/80. Carotid sinus pressure, eyeball pressure and vomiting induced by ipecac failed to influence the heart rate. The electrocardiogram revealed the presence of 1:1 atrial flutter. (Fig. 6A.) Cedilanid was administered intravenously, 0.8 mg. shortly after admission followed by 0.4 mg. every four hours for four doses. Following the last dose the rhythm became irregular and averaged about 100 per minute. An electrocardiogram revealed the presence of atrial fibrillation. (Fig. 6B.) The patient was greatly improved. The blood pressure was 140/80. A daily maintenance dose of 0.3 mg. of digitoxin was then prescribed. On the third day of hospitalization the heart rate was regular at 60 per minute. An electrocardiogram revealed normal sinus rhythm and digitalis effect. (Fig. 6C.)

Although the patient was fully digitalized, he had two more attacks of 1:1 atrial flutter while in the hospital. Confirmed by electrocardiogram, they were of short duration and were promptly controlled by intravenous injection of 600 mg. and 400 mg. of pronestyl,® respectively. In each instance the atrial flutter was converted directly to normal sinus rhythm.

Routine laboratory studies were within normal limits; the serum cholesterol was 184 mg. per cent; cholesterol esters were 112 mg. per cent; radioactive iodine uptake was 50 per cent in twenty-four hours

and 49 per cent in forty-eight hours; protein-bound iodine was 8.2 μg. (normal value for this hospital 3.7 to 6.7); urinary creatine was 277 mg. per 1,900 cc. of urine while the creatinine was 988 mg. per 1,900 cc. urine. Chest and neck x-rays revealed no abnormalities. A presumptive diagnosis of thyrotoxicosis was made and the patient was given propylthiouracil. He was discharged improved on February 2, 1952 on a maintenance regimen of digitoxin and propylthiouracil.

COMMENTS

Although 1:1 atrial flutter is usually encountered in association with serious myocardial damage, it is of interest that two of our six patients had no "clinically" detectable heart disease and that the autopsy findings in one who died after a second bout of tachycardia failed to show evidence of pre-existing cardiac pathology. In the remainder of our series, evidence of rheumatic heart disease was noted in one patient, arteriosclerotic hypertensive disease in two, and probable thyrotoxic heart disease in one patient.

Paroxysms of 1:1 flutter may be precipitated by excitement, exertion, emotional strain or any state associated with increased sympathetic tone in susceptible persons. It may also occur during quinidine treatment of atrial flutter when the atrial rate is slowed by the drug and the ventricle responds to each auricular impulse.[25,31] Atropine has also been implicated in the production of 1:1 flutter when given intravenously to patients with rheumatic heart disease, auricular fibrillation or flutter with 2:1 block.[22] In our series the onset of tachycardia was related to induction of anesthesia in one patient, in two others it followed exertion and excitement.

When 1:1 atrial flutter occurs, it is most frequent in the fifth decade of life. It has not been reported above the age of sixty although 2:1 or 3:1 flutter is frequently seen even after the age of seventy.[26] This may be the result of degenerative changes in the A-V node which develop with increasing age and which do not permit the rapid (1:1) transmission of auricular impulses across the junctional tissues. The age of our patients varied from sixteen to sixty years.

The sex distribution of atrial flutter in general favors the male in a ratio of 4:1; this holds true for the reported cases of 1:1 flutter. In our small series both sexes were equally represented.

The reported duration of the paroxysms of 1:1 flutter varied from a few minutes to two or three hours. In our cases tachycardia lasted from a few minutes to over five days. In our first patient, who had had two bouts of 1:1 flutter, the first attack was finally broken up after five days, while the second, which eventuated in death, lasted over five days before there was an increase in the degree of A-V block with resultant slowing of the ventricular rate.

The symptoms and signs of 1:1 flutter depend on the age of the patient, the ventricular rate, the duration of the tachycardia, and the presence or absence of previous cardiac disease. In none of our cases was the flutter associated with development of myocardial infarction, although this diagnosis was entertained in some instances because of the presenting symptoms.

Duration of the tachycardia bears an important relationship to the prognosis in a given paroxysm of 1:1 flutter. If long lasting, it produces irreversible changes in the cardiovascular system. This is well illustrated by the patient in our Case 1 who died, notwithstanding conversion to normal sinus rhythm, after the 1:1 flutter had lasted a little over five days. Although she was a young girl of sixteen and had had no "clinically" demonstrable heart disease, irreversible changes had occurred as a result of persistent tachycardia.

In one of our patients a cerebrovascular accident developed with hemiplegia nine days after conversion to normal sinus rhythm but unfortunately a necropsy was not obtained.

Hejtmancik et al.[26] have emphasized the fact that embolism is a rare occurrence during atrial flutter. When it is observed the embolism usually occurs after conversion of the flutter into atrial fibrillation or sinus rhythm. The lesser incidence of embolism during flutter as compared to atrial fibrillation has been attributed to the fact that in flutter the auricles display regular, coordinated contractions.

The diagnosis of 1:1 flutter may be suspected clinically; it can be definitely established only by serial electrocardiograms taken before, during and/or after therapy. The following electrocardiographic findings are worthy of emphasis: (1) The characteristic regular undulating wave of atrial flutter may not be seen in all leads of the electrocardiogram; its appearance depends on the relationship of the direction of the atrial vector to the lead axis. In our series it was best seen in leads II, III, aVF and the right precordial leads V_1 and V_3R. (2) The atrial rate ranges from 225 to 370 with an average of 300 per minute.[30] (3) The duration of the QRS complex may be within normal limits or prolonged. The

latter may be the result either of pre-existing heart disease with established bundle branch block or of functional fatigue of the bundle branches secondary to the rapid rate. The widening of the QRS complex disappeared in our cases upon resumption of sinus rhythm, indicating the reversible nature of the intraventricular conduction defect. (4) Carotid sinus pressure is usually ineffective in slowing the ventricular rate.[11] It was attempted repeatedly in our patients but was found ineffective except in one patient who had been receiving digitalis, which increased the vagal sensitivity. In contrast, in atrial flutter with higher degrees of A-V block our experience has been that carotid sinus pressure or other means of vagal stimulation usually results in a further though temporary increase in the degree of A-V block. (A 2:1 flutter is thus converted into 3:1, 4:1 or higher.) (5) The esophageal lead at some atrial level reveals the presence of an intrinsicoid P wave with absence of isoelectric P-P intervals. Multiple esophageal leads at various atrial levels must be obtained since the broad ascending P-P line that starts before the end of one P wave and terminates at the beginning of the succeeding P wave may be seen only at certain atrial levels. Its presence serves to distinguish flutter from atrial tachycardia.[28]

The electrocardiographic diagnosis of 1:1 flutter may not be clear cut. In such cases prior evidence of atrial flutter with 2:1 or higher degrees of A-V block strongly favors the diagnosis of 1:1 flutter; this is further confirmed by the development either of higher grades of A-V block or of atrial fibrillation following digitalis therapy.

Atrial flutter with 1:1 A-V conduction must be differentiated from paroxysmal atrial, nodal and ventricular tachycardia. In most cases the difference in rate between these arrhythmias helps to establish a correct diagnosis. However, an occasional case of supraventricular or ventricular tachycardia may display a rate similar to that of 1:1 flutter. The following points help in the differential diagnosis: in adults the rate of supraventricular tachycardia ranges from 140 to 220 per minute. The application of carotid sinus pressure or other means of vagal stimulation (prostigmin, digitalis, beta-methylcholine, emetic drugs, and the like) usually results in sudden cessation of the paroxysm and halving of the cardiac rate with the return of sinus rhythm. The electrocardiogram shows an isoelectric period between the P waves; if the P wave cannot be identified in the standard leads or right precordial leads (over the right atrium) then esophageal leads taken at various atrial levels will demonstrate the atrial complex (intrinsicoid P wave).

The presence of nodal tachycardia can be established by the shortened P-R interval of less than 0.12 second, by the tendency for the pacemaker to wander within the A-V node, by the presence of an inverted P wave that follows the QRS complex whenever the ectopic focus is located at the lower end of the A-V node and, finally, by exercise or full atropinization which may (by abolishing vagal tone) help separate the P wave from the QRS complex and thus assist in identification of the basic nodal rhythm.

In the common type of ventricular tachycardia, the ventricular rate ranges from 130 to 180 per minute. Carotid sinus pressure is ineffective in slowing the rate. The electrocardiogram displays slight irregularity of the R-R time while the atria beat at a slower rate and independently of the ventricles, and finally the QRS complexes are widened, slurred, notched and bizarre. Esophageal leads are of great value in establishing the diagnosis.

Our experience and that of others indicates that digitalis is the drug of choice in the treatment of atrial flutter with 1:1 A-V conduction. After adequate therapy, either the degree of A-V block increases or the mechanism changes from flutter to atrial fibrillation. The grave implications of the rapid ventricular rate in 1:1 flutter make rapid digitalis therapy by intravenous or intramuscular routes almost mandatory. Five of our patients received the drug intravenously; in three, ouabain was used, while two patients received cedilanid.

Of the six patients treated with digitalis, the mechanism changed directly from flutter to atrial fibrillation in four. One patient displayed atrial flutter with a higher degree of A-V block before it changed to atrial fibrillation, and the sixth patient continued to display atrial flutter with a high degree of A-V block. Of the patients who were converted to atrial fibrillation, four reverted spontaneously to normal sinus rhythm; in one, quinidine was administered in order to restore normal sinus rhythm.

We have repeatedly confirmed the observation[11,11b,30] that cases of established atrial flutter with higher degrees of A-V block (2:1, 3:1, and the like) frequently require larger than usual

digitalis dosage in order to alter or abolish the arrhythmia. In contrast, from the results obtained in five of our six cases it would appear that in the presence of 1:1 atrial flutter an average digitalis dose is adequate to obtain either a higher degree of A-V block or conversion to atrial fibrillation or normal sinus rhythm. The sixth patient (Case I) proved to be resistant to very large doses of digitalis administered by various routes.

When digitalis fails to affect the arrhythmia, quinidine may be used. One of our patients (Case I) received quinidine repeatedly during two bouts of 1:1 atrial flutter, without effect except for a temporary slowing of both the atrial and ventricular rates. On one occasion the paroxysm was terminated by rapid intravenous injection of quinine dihydrochloride. Quinidine was also used in one of our patients (Case III) to restore normal sinus rhythm after the 1:1 flutter had been converted by digitalis to atrial fibrillation.

We have successfully used procaine amide (pronestyl) in the treatment of two paroxysms of 1:1 flutter in one of our patients. (Case VI.) However, Bellet[30] has found this drug to be inferior to quinidine in the treatment of established atrial flutter with higher degrees of A-V block. It restored normal sinus rhythm in only three of seventeen such cases; the dose required was usually large and may be within the toxic range.

Neo-synephrine® has been used in a case of 1:1 flutter with success.[29] This drug acts through reflex vagal stimulation secondary to the resulting hypertension. However, neo-synephrine is not dependable and may actually be dangerous because of its direct stimulating effect on the myocardium during a period when the heart is already under increased stress.

SUMMARY

1. Our experience in the diagnosis and treatment of six cases of atrial flutter with 1:1 A-V response is reviewed.

2. In the six patients, the paroxysms of 1:1 atrial flutter were controlled by therapy; one patient died as a result of congestive heart failure and, at autopsy, the heart failed to show any significant lesions.

3. Although 1:1 atrial flutter is rare, its occurrence requires emergency treatment because of the shock-like state that usually ensues. Its presence should be suspected in patients with or without clinically detectable heart disease if a tachycardia of 225 to 315 beats per minute develops suddenly and if carotid sinus pressure fails to slow the rate. The diagnosis may be established by means of the electrocardiogram taken before, during and/or after treatment. The diagnostic criteria are presented.

4. Digitalis is the drug of choice in the treatment of this arrhythmia. Because of the critical state of the patient, it should be administered parenterally, preferably by vein. Quinidine and procaine amide may be of value in the occasional patient when digitalis fails.

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Chronic Poliomyelitic Respirator Deaths*

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MOST deaths due to poliomyelitis occur during the acute phase of the disease. Better understanding of the pathologic physiology and improvement in treatment technics have appreciably reduced the mortality rate. If we consider only those patients whose condition was serious enough to require tracheostomy or respiratory aid, the mortality rate will be more meaningful and will indicate the severity of the disease and the efficiency of treatment. On the other hand, a mortality rate based on all reported cases is more apt to reflect the proportion of mild or non-paralytic cases.

Counting only the serious cases, the mortality rate varies from 13 to 50 per cent from one community to another. An average figure is about 20 per cent whereas only a few years ago it was 50 to 75 per cent. The greater survival has resulted in an accumulation of severely paralyzed, permanent or semi-permanent respirator patients. Although there are a few examples of respirator patients who are still living twenty or more years after onset, experience with large numbers of postacute respirator patients is relatively short.

Those directly or indirectly concerned with poliomyelitis frequently inquire about the prognosis of a chronic respirator patient and the complications that can be expected. Because of the frequency of such questions, it was considered pertinent to analyze the deaths and autopsy findings in chronic respirator patients treated at this respirator center.

From January 1, 1952, to July 15, 1954, a period of thirty-one months, there were approximately 585 chronic poliomyelitic respirator patients treated at this hospital. All patients were admitted two weeks or more after onset of disease, having been treated elsewhere during that phase. Some were readmissions from their homes because of medical complications. All were supposed not to be in the acute state, although some failed to show clinical improvement and followed a continuous downhill course culminating in death. During this thirty-one months there were fifteen deaths, a mortality rate of 2.05 per cent. Necropsy studies were made in all fifteen patients. (Table I.) An abstract of each case, with cause of death as determined at necropsy, will follow. More detailed summaries of necropsy findings are separately tabulated.

ABSTRACTS OF CASE HISTORIES AND AUTOPSY REPORTS

CASE I. M. B., a thirty-one year old white woman with bulbospinal paralytic poliomyelitis, was transferred to Rancho Los Amigos two months after onset and remained two months until death. Tracheostomy was performed, a tank respirator was employed full time and a Levin tube was used for feeding. This patient was severely emaciated and from the time of admission was considered to have a poor prognosis. During the final six weeks she suffered several episodes of acute abdominal distention and had excessive tracheobronchial mucus. Tracheal cultures demonstrated Proteus vulgaris which was insensitive to tested antibiotics (streptomycin, penicillin, terramycin,® aureomycin and polymyxin). The patient died without showing physical signs other than copious mucus and continuation of a low grade fever to indicate underlying pathologic processes. At necropsy confluent, bilateral bronchopneumonia with multiple small lung abscesses was noted. Multiple gastric and esophageal ulcers were found. The sphenoid sinus showed purulent sinusitis.

CASE II. R. P., a nine year old white boy with bulbospinal paralytic poliomyelitis, had left

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TABLE I

CHRONIC POLIOMYELITIS, POSTMORTEM EXAMINATIONS

VITAL STATISTICS

Patient	Sex and Age (yr.)	Type of Poliomyelitis	Duration	Cause of Death
M. B.	F, 31	Bulbospinal	4 mo.	Bronchopneumonia, multiple lung abscesses
R. P.	M, 9	Bulbospinal	3 yr., 5 mo.	Gastric hemorrhage, diffuse gastritis
W. F.	F, 29	Bulbospinal	9 mo.	Bronchopneumonia, lung abscesses with extension
T. S.	F, 16	Bulbospinal	12 mo.	Bilateral atelectasis, bronchopneumonia
D. P.	M, 28	Bulbospinal	4 mo.	Pyloric obstruction, lung abscess, bronchopneumonia
B. M.	F, 29	Bulbospinal	4 mo.	Massive atelectasis, pulmonary edema
E. M.	F, 40	Bulbospinal	4 yr., 4 mo.	Bilateral spontaneous pneumothorax
D. L.	F, 30	Spinal	7 mo.	Peritonitis, gastric hemorrhage, gastritis
S. B.	M, 7	Bulbar	7 mo.	Tracheal obstruction, polyp
J. H.	F, 28	Bulbospinal	8 mo.	Ruptured appendix, peritonitis
E. C.	F, 24	Bulbospinal	7½ mo.	Pulmonary hemorrhage, bronchiectasis
L. E.	F, 30	Bulbospinal	6 mo.	Lung abscess, purulent pericarditis
M. V.	F, 25	Bulbospinal	9 mo.	Massive pulmonary abscesses
W. Y.	F, 28	Bulbospinal	1 yr., 3 mo.	Pyonephrosis, perinephric abscess and peritonitis
B. G.	F, 6	Bulbospinal	1 yr., 10 mo.	Acute gastric distention and jejunal intussusception

lower lobe atelectasis during the acute phase of the disease. This patient arrived at Rancho Los Amigos twelve months after onset. Tracheostomy was performed and a tank respirator was used full time. There was extensive involvement of all extremities, intercostal muscles and diaphragm. During the three and one-half-year period from admission to death he showed little progress and his stay was punctuated by occasional upper respiratory infections and episodes of acute abdominal distention. During the last three months he had persistent occult blood in his stools. Little diagnostic work could be done because of the patient's poor respiratory status. He died three years and five months after onset of his acute disease during a severe episode of upper gastrointestinal bleeding. At necropsy it was found that he had massive gastric hemorrhage due to diffuse superficial gastric ulceration and multiple esophageal ulcers.

CASE III. W. F., a twenty-nine year old white woman with bulbospinal paralytic poliomyelitis, had a stormy course during the acute phase of the disease, with convulsive seizures, pneumonia and atelectasis. Cultures of the tracheobronchial secretions, as well as urine cultures, demonstrated Pseudomonas aeruginosa. She was transferred to Rancho Los Amigos two and one-half months after onset. A tank respirator was in use full time and tracheostomy had been performed. A Levin tube was necessary for feeding. The patient was wasted and emaciated, and the prognosis was poor. Her course was uneventful until six months after admission when convulsive seizures again developed. There was associated heavy tracheobronchial mucus and gross rectal hemorrhage without a febrile response or any other signs of infection. She died two weeks after the convulsions. At necropsy confluent bronchopneumonia with multiple lung abscesses was

found. The right pleural cavity had a localized abscess involving the lung and diaphragm. There was also a subphrenic abscess with hepatic subcapsular abscess formation. Cultures from all these sites demonstrated Ps. aeruginosa.

CASE IV. T. S., a sixteen year old white girl, had acute bulbospinal paralytic poliomyelitis punctuated by two episodes of atelectasis and numerous episodes of acute abdominal distention. She was transferred to Rancho Los Amigos three months after onset; she had had tracheostomy and was in a tank-type respirator. Her early course at Rancho Los Amigos was stormy with acute abdominal distention. Four months after onset a temperature of 107°F. (rectal) developed with severe cephalalgia and ensuing generalized convulsions. She was considered to have acute encephalitis of unknown etiology but presumably related to the poliomyelitis. The following day she lapsed into semi-coma in which she remained until her death nine months later. During this entire period she had profuse tracheobronchial mucus and required frequent suctioning and close nursing attention. At necropsy the patient was found to have bilateral atelectasis with patchy areas of bronchopneumonia, and chronic pyelonephritis with renal calculi and calcinosis.

CASE V. D. P., a twenty-eight year old white man with acute bulbospinal paralytic poliomyelitis, suffered thrombophlebitis of the left common femoral vein which required ligation and thrombectomy because of local symptoms and pulmonary embolism. Upon arrival at Rancho Los Amigos two months after onset he was using a tank respirator. During the ensuing few months the patient progressed to the use of the cuirass respirator and rocking bed. Shortly thereafter laryngeal edema and acute pyloric obstruction with abdominal distention suddenly developed. Emergency tracheostomy was performed. From this time on his course was rapidly downhill. The pyloric obstruction, which required Wangensteen suction and electrolyte restorative therapy, continued until death two weeks later. A polyethylene tube was inserted in the antecubital vein for continuous feeding. Thrombophlebitis of the axillary vein developed, with anterior chest wall edema. The patient died two months after admission with an overwhelming infection, despite antibiotic and restorative therapy. At necropsy bronchopneumonia with lung abscesses was found from which P. vulgaris was cultured. A left anterior chest wall abscess was noted with associated thrombophlebitis of the cephalic and basilic veins. There were esophageal ulcerations as well as extensive superficial gastric ulceration. Small embolic abscesses of the kidney parenchyma and myocardium were also found.

CASE VI. B. M., a twenty-nine year old white woman with bulbospinal paralytic poliomyelitis, had a stormy course from onset, with heavy tracheal mucus from which Ps. aeruginosa was cultured. The patient was transferred to Rancho Los Amigos three months after onset; tracheostomy had been performed and she was in a tank respirator. A Levin tube was required for feeding. From the time of her arrival she exhibited copious thick yellow mucus from the trachea which cultured Ps. aeruginosa, P. vulgaris and Escherichia coli. Chest x-ray disclosed atelectasis which was treated with antibiotics, with clinical response. One month after admission generalized convulsions developed with tachycardia and associated blood-tinged sputum. Bright blood was aspirated from the Levin tube. She died twenty-four hours after the onset of these symptoms. At necropsy severe atelectasis with associated bronchopneumonia was found. This was complicated by severe, massive pulmonary edema, renal calculi and chronic pyelonephritis.

CASE VII. E. M., a forty year old white woman with bulbospinal paralytic poliomyelitis, had spent several years at home in a tank-type respirator. She was admitted to Rancho Los Amigos during an acute episode of tracheobronchitis and bronchopneumonia. Tracheostomy had been performed and the patient was using the tank respirator full time. Upon recovery the cannula was removed. Several weeks thereafter thick copious mucus again developed which she was able to raise with the aid of the respirator. A few nights later extreme pallor with stridulous respiration suddenly developed. A second tracheostomy was immediately performed moments before death. No mucus was obtained by catheter suctioning. At necropsy bilateral pneumothorax was found which was apparently spontaneous in nature. There was also evidence of acute bronchitis and bilateral renal calculi.

CASE VIII. D. L., a thirty year old white woman with spinal paralytic poliomyelitis, was transferred to Rancho Los Amigos in a tank-type respirator; tracheostomy had been performed. Her course was punctuated by

numerous episodes of pulmonary infection complicated on several occasions by atelectasis. She had severe hypertension which responded to apresoline.® Seven months after onset her final episode was initiated by gastric distention and abdominal pain. She required a Levin tube with gastric suction for decompression. The gastric contents contained a large amount of bright blood, lasting for several days. She lapsed into shock with a rapid downhill course and died despite all restorative measures. At necropsy a localized area of peritonitis was found in the area of the lesser omental sac. This was associated with massive, hemorrhagic gastritis. There were numerous superficial esophageal ulcers noted, as well as cholelithiasis and chronic pleuritis.

Case IX. S. B., a seven year old white boy with bulbar paralytic poliomyelitis, was transferred to Rancho Los Amigos in a tank respirator; tracheostomy had been performed. He was removed from the respirator in a short time. His course was uneventful and six months after onset the cannula was removed. The next day was uneventful; however, in the early evening of the second day he experienced minor intermittent respiratory difficulty. That night a convulsive seizure with cyanosis suddenly developed and he died within two minutes. The tracheostomy site was reopened under emergency conditions and artificial respiration administered without success. At necropsy a polyp was found obstructing the trachea. The polyp was intraluminal at the cephalad aspect of the tracheostoma. It apparently occluded the trachea only after edema and inspissated mucus had reduced the lumen.

Case X. J. H., a twenty-eight year old white girl, had an acute onset of bulbospinal paralytic poliomyelitis complicated by a recent pregnancy; her two months old infant also showed signs of poliomyelitis, spinal in type.

The patient was transferred to Rancho Los Amigos one month after onset. She was using a tank-type respirator and a Levin tube for feeding, and had had a tracheostomy. It was noted on admission that a microcytic, hypochromic anemia existed. This was interpreted as a blood-loss type of anemia. Occult blood was repeatedly found in the stool but no gross bleeding was evident. She required multiple transfusions. Eight months after onset convulsive seizures and coma developed. She was afebrile and had no signs of a complicating disease process. Four days later, while still in coma, a high fever with abdominal distention developed. The stomach contained coffee-ground material. She died the next day. At necropsy a ruptured appendix with localized abscess formation was noted.

Case XI. E. C., a twenty-four year old white woman with bulbospinal paralytic poliomyelitis, was transferred to Rancho Los Amigos three months after onset. She was using a tank-type respirator and a Levin tube for feeding, and had had tracheostomy. Seven and one-half months after onset hemorrhagic mucus from the tracheostomy site as well as from the nose suddenly developed. The Levin tube was removed and the nose packed; however, the hemorrhagic continued. Within twenty-four hours she had a sudden convulsive seizure and died minutes thereafter. At necropsy massive pulmonary hemorrhage was found, originating in a granulomatous bronchiectatic area of the lung. The pericardium had a contiguous extension from the granulomatous lung lesion, with resulting abscess formation. Purulent ethmoid and sphenoid sinusitis was noted and cultured. All sites cultured Ps. aeruginosa and P. vulgaris. Microscopic study disclosed lipoid pneumonia superimposed upon bronchiectasis.

Case XII. L. E., a thirty year old white woman, had acute bulbospinal paralytic poliomyelitis complicated by a third trimester intrauterine pregnancy which was delivered while the patient was in respiratory equipment. She was transferred to Rancho Los Amigos three months after onset in a tank-type respirator; tracheostomy had been performed. On admission a rather severe anemia was noted. Chest x-ray showed an enlarged, globular-shaped heart. Electrocardiogram demonstrated evidence of myocardial damage. The patient had subjective complaints of pain in the anterior chest and an elevated temperature. Objectively there was 4 plus pitting edema of the entire body up to the clavicular region. A diagnosis of congestive heart failure, obscure etiology, was made and therapy instituted. An irregular, low grade fever continued despite antibiotics. The patient became semi-comatose and died six months after onset of poliomyelitis. At necropsy suppurative pericarditis with associated pleuritis was noted, as well as a solitary lung abscess and numerous embolic abscesses of the kidneys. Massive atelectasis secondary to bilateral pleural effusion was associated with other evidences of

congestive heart failure. Cultures of suppurative areas showed Ps. aeruginosa.

CASE XIII. M. V., a twenty-five year old white woman with bulbospinal paralytic poliomyelitis, had copious tracheal secretions during the acute phase of the disease. Upon arrival at Rancho Los Amigos two and one-half months after onset tracheostomy was performed and a tank respirator was used full time. This patient required a Levin tube for feeding, and almost from arrival manifested varying degrees of pulmonary consolidation which progressed throughout the remaining six and one-half months of her life despite antibiotic therapy and bronchoscopy with tryptar® instillation. Near the end of this period the patient experienced several pulmonary hemorrhages and there was x-ray evidence of abscess formation complicating her atelectasis and pneumonia. At necropsy massive atelectasis with pulmonary abscess formation was found from which Ps. aeruginosa and Micrococcus (Staphylococcus) aureus, coagulase-positive, were cultured.

CASE XIV. W. Y., a twenty-eight year old white woman with bulbospinal paralytic poliomyelitis, suffered numerous episodes of cyanosis during the acute phase of the disease. These were usually associated with bronchial mucous plugs and periods of irrationality. She was transferred to Rancho Los Amigos two and one-half months after onset. Tracheostomy had been performed and she was using a tank respirator. From the time of admission this patient suffered with persistent chronic atelectasis and associated air hunger. The additional complication of bilateral renal calculi subsequently developed, with repeated acute pyelonephritic attacks superimposed upon chronic smoldering pyelonephritis. These continued to the time of her death twelve months later. P. vulgaris and Escherichia coli were repeatedly cultured from the urine and were refractory to therapy because of poor drainage incident to the renal calculi. The patient's condition was never good enough to consider renal surgery. She followed a downhill course and terminally suffered acute gastric distention with vomiting and electrolyte imbalance. At necropsy purulent peritonitis with bowel dilatation was found, apparently secondary to a perinephric abscess subsequent to pyonephrosis and multiple renal cortical abscesses. Multiple renal stones were found in the major and minor calyces, pelvis and urinary bladder.

CASE XV. B. G., a six year old white girl with bulbospinal paralytic poliomyelitis, had a stormy course during the acute phrase of the disease and was transferred to Rancho Los Amigos one month after onset. Tracheostomy had been performed and she was using a tank respirator. This patient refused to eat and suffered from poor nutrition complicated by frequent episodes of gastric distention. She suffered several episodes of upper lobe atelectasis which responded to therapy, clearing completely. One year and nine months after admission this patient was considered stabilized, requiring part time respiratory aid. She was transferred to the "Home Care" program (sent home under the care of her parents). Three weeks after discharge she was returned following a convulsion, with the story that early in the day she had complained of a stomach ache and vomited. She was pronounded dead on arrival. At necropsy massive gastric dilatation was noted, complicated by numerous agonal jejunal intussusceptions. These were considered a terminal complication because of the lack of reaction. Renal stones and chronic pyelonephritis were noted.

COMMENTS

The preceding case reports demonstrate the frequency of involvement of several organ systems.

Respiratory System. As one would expect, because of the nature of the basic disability, the respiratory system is frequently affected.[3] (Table II.) Bronchopneumonia, a common clinical complication in the respirator patient, directly resulted in death in four of the patients herein described and was present as a contributing cause in five additional patients. Bronchopneumonia was accompanied by complicating lung abscesses in five cases. One patient died as a direct result of lung abscess with extension. Atelectasis was another equally prevalent pulmonary complication, seen all too frequently clinically.[1,2] This was noted in ten of our patients, either associated with pneumonia or as the immediate cause of death, as in two cases. Strauss and Bluestone,[5] in one of the few reports in the literature of chronic respiratory deaths with necropsy, stated that their patient died five months after cesarean section while confined to respiratory equipment and showed atelectasis with mediastinal shift, pyelonephritis and renal calculi. The microorganisms isolated from the necropsy studies almost without exception

TABLE II

CHRONIC POLIOMYELITIS, POSTMORTEM EXAMINATIONS

RESPIRATORY SYSTEM

Findings	M. B.	R. P.	W. F.	T. S.	D. P.	B. M.	E. M.	D. L.	S. B.	J. H.	E. C.	L. E.	M. V.	W. Y.	B. G.	No.	Per cent
Atelectasis:																	
Gross	−	+	−	+	−	+	+	−	−	+	+	+	+	+	+	10	67
Microscopic	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	14	93
Emphysema:																	
Gross	−	−	+	+	−	−	+	−	−	+	−	−	−	−	+	5	33
Microscopic	−	+	+	+	−	−	+	+	−	+	−	−	−	+	+	8	53
Abscesses:																	
Without pneumonitis	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	1	7
Organisms	−	−	−	−	−	−	−	−	−	−	−	PA,* FB†	−	−	−	−	...
Gross pneumonitis	+	−	+	+	+	+	−	+	−	−	+	−	+	+	−	9	60
Microscopic pneumonitis:																	
With abscess formation	+	−	+	−	+	−	−	−	−	−	+	−	+	−	−	5	33
Without abscess formation	−	−	−	+	−	+	−	+	−	−	−	−	−	+	−	4	27
Pneumonitis organisms	PV†	−	PA	−	PV	−	−	−	−	−	PA	−	PA	−	−	−	...
Hyaline alveolar membranes	−	+	−	+	+	−	+	+	−	−	−	+	−	+	−	7	47
Tracheostomy:																	
Presence	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	100
Ulceration	−	−	−	−	+	−	−	+	+	+	−	−	−	−	−	4	27
Evidences of chronic trauma	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	2	13

* PA = Pseudomonas aeruginosa.

† FB = Friedländer's bacillus.

‡ PV = Proteus vulgaris.

proved to be either P. vulgaris or Ps. aeruginosa.[21] The same microorganisms show themselves time and again in our tracheobronchial mucous aspiration cultures. Needless to say, these microorganisms have been most recalcitrant to our efforts at eradication. Moreover, they have demonstrated an unhappy faculty for contiguous spread, with sinus tract formation and all the resulting secondary complications encountered in our series. Suppurative pleuritis, pericarditis, diaphragmatic abscesses, hepatic subcapsular and hepatic parenchymal abscesses were such complications in three of our cases.

These patients all received penicillin prophylactically during their acute phase, but after admission to this hospital received antibiotics only for a specific infection. This naturally raises a question as to the advisability of using antibiotics for prophylactic purposes in these patients. However, the limited number of patients in this study does not permit drawing any conclusions.

Clinically, it is often difficult to diagnose early pneumonia or atelectasis because of the poor physical response exhibited by the patient. In several of our cases minimal or no temperature response was exhibited with the inception and progress of pneumonia. Yow,[21] in his paper on Proteus and Pseudomonas, makes a point of the lack of temperature response in the presence of severe infections with these organisms.

Purulent sinusitis is another equally troublesome complication seen with prolonged use of the nasal Levin tube for feeding. The same offending microorganisms have been most frequently isolated. Frequent tube changes with alternation of nares has been found to reduce appreciably the prevalence of this condition.

A thorough study of the cases revealed five with gross evidence of emphysema and three additional ones demonstrating microscopic evidences of emphysema. Only one of these patients suffered readily demonstrable ill effects; namely, spontaneous pneumothorax. Some of the most severely involved patients, those requiring longest residence in respiratory equipment, manifest the most severe emphysematous involvement. (Table II.)

An additional interesting but poorly understood microscopic finding was the presence in six patients of an irregular layer of homogeneous acidophilic material in intimate association with the inner surfaces of the alveolar ducts. This material is similar in appearance to that seen in the lungs of newborns. This specific condition has been admirably summarized by Potter[25] who expresses the belief that hyaline membrane forms a mechanical barrier to normal respiratory exchange. The exact chemical composition of this substance and its origin are not definitely established. These same "hyaline membranes" have been seen in pneumonia associated with rheumatic disease and in fatal chicken pox.[25] They have also been described in fatal measles and influenza,[24] and have been seen in acute poliomyelitic deaths. This complication may well represent a barrier to normal gas exchange. Further study of this problem is underway.

It will be noted that all of our patients had tracheostomies, and that one suffered a fatal complication at the time of closure due to obstruction by a polyp. This polyp was inflammatory in origin and was essentially granulation tissue. Similar polyps have been found in four additional cases, fortunately before such an unfortunate episode occurred. A routine examination through the tracheostomy stoma with a small nasopharyngoscope is carried out on all patients in whom decannulation is anticipated.

At necropsy the trachea of each patient was studied carefully for evidence of trauma incident to long use of the tracheostomy cannula and repeated aspiration of secretions via this stoma. Areas of thickened mucosa on the posterior tracheal wall opposite the cannula are seen rather consistently. Small superficial mucosal ulcerations, petechial hemorrhages or localized areas of erythema have all been considered to be evidence of mechanical chronic trauma incident to repeated aspiration with a soft rubber catheter.

Cardiovascular System. The cardiovascular system rarely contributed directly to any deaths, with the possible exception of Case XII (Table III) in which congestive failure occurred complicating lung abscesses with contiguous spread to the pericardium and resultant myocarditis.

Close study of the hearts disclosed three cases with dilatation of all chambers and two additional ones with minimal dilatation. Six cases showed frank right ventricular hypertrophy and two showed slight hypertrophy.

Eleven patients were found to have left ventricular hypertrophy as evidenced by observation, ventricular wall thickness and total heart weight. These criteria were interpreted in the light of patient's size and predicted normal. How this cardiac hypertrophy develops is not

TABLE III

CHRONIC POLIOMYELITIS, POSTMORTEM EXAMINATIONS

CARDIOVASCULAR SYSTEM

Findings	M. B.	R. P.	W. F.	T. S.	D. P.	B. M.	E. M.	D. L.	S. B.	J. H.	E. C.	L. E.	M. V.	W. Y.	B. G.	No.	Per cent
Cardiac dilatation:																	
Right ventricular	−	−	−	+	+	+	−	+	−	−	−	+	−	+	−	6	40
Left ventricular	−	−	−	+	+	+	−	+	−	−	−	+	−	−	−	5	33
Cardiac hypertrophy:																	
Right-sided	−	−	+	+	+	+	−	−	−	−	+	+	+	+	−	8	53
Left-sided	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	11	73
Myocarditis:																	
Gross	−	−	−	−	+	−	−	−	−	−	−	+	+	−	+	4	27
Microscopic	+	−	+	+	+	−	+	−	−	−	+	+	+	−	+	9	60
Pericarditis	+	−	+	−	−	+	−	−	−	−	+	+	+	+	−	7	47
Organisms	PV	−	−	−	−	−	−	−	−	−	PA	PA, FB	−	−	−	3	20

TABLE IV

CHRONIC POLIOMYELITIS, POSTMORTEM EXAMINATIONS

GASTROINTESTINAL SYSTEM

Findings	M. B.	R. P.	W. F.	T. S.	D. P.	B. M.	E. M.	D. L.	S. B.	J. H.	E. C.	L. E.	M. V.	W. Y.	B. G.	No.	Per cent
Ulcerations:																	
Esophagus	+	+	−	−	+	−	−	+	−	−	+	+	−	−	−	6	40
Stomach	+	+	−	−	+	−	−	+	−	−	−	−	−	+	+	6	40
Duodenum	−	−	−	+	.....	−	−	−	−	−	−	−	−	−	−	1	7
With hemorrhage	+	+	−	+	+	−	−	+	−	−	−	−	−	+	+	7	47
Abscess formation	−	−	+	..	+	−	−	+	−	+	−	−	−	−	−	4	27
Site	−	−	Liver	..	Esophagus	..	−	Stomach	−	Appendix	−	−	−	−	−	..	..
With perforation	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	2	13
Gastritis	−	−	+	−	−	−	−	−	−	+	+	+	−	+	+	6	40
Peritonitis	−	−	−	−	−	−	−	+	−	+	−	−	−	+ PV, EC*	−	3	20

* EC = Escherichia coli.

TABLE V
CHRONIC POLIOMYELITIS, POSTMORTEM EXAMINATIONS
GENITOURINARY SYSTEM

Findings	M. B.	R. P.	W. F.	T. S.	D. P.	B. M.	E. M.	D. L.	S. B.	J. H.	E. C.	L. E.	M. V.	W. Y.	B. G.	No.	Per cent
Calculi	−	+	−	+	+	+	+	+	+	+	+	+	−	+	+	12	80
Kidney pelvis	−	+	−	+	−	+	+	+	+	+	−	+	−	+	+	10	67
Bladder	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	3	20
Hydroureter	+	−	−	+	−	−	−	−	−	−	−	+	−	+	−	4	27
Infection:																	
Pyelonephritis	−	+	−	+	+	+	−	+	+	+	−	+	−	+	−	10	67
Cystitis	+	−	+	−	+	−	−	−	+	+	+	−	−	+	−	7	47
Hypertensive changes:																	
Clinical	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	2	13
Gross	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0	0
Microscopic	+	−	−	−	−	+	+	+	+	−	−	−	−	−	−	5	33

clear and just what part residence in respiratory equipment plays is not known.

Two patients in our series showed clinical evidence of hypertension, with a satisfactory response to antihypertensive drugs. A number of cases with episodes of rather marked hypertension have been seen; all, over a period of time, have responded to antihypertensive drugs.

A microscopic search for myocarditis, using essentially the criteria of Saphir and Wile,[6] and Teloh,[7] revealed eight cases with microscopic changes, of which four had grossly evident lesions. Three additional patients had microscopic lesions very suggestive of healed myocarditis. Since this incidence is far above the anticipated general population frequency, at least some of the cases can be ascribed to poliomyelitis-induced myocarditis. (Table III.)

The vascular thrombosis with resultant visceral infarction seen in two cases was secondary to microorganism contamination, one case following use of an intravenous polyethylene tube and the other following spread of lung abscesses.

Gastrointestinal System. The gastrointestinal system is frequently the site of symptoms clinically and had its share of complications in our series. (Table IV.) Abdominal distention with signs of gastrointestinal bleeding was noted during life in eleven patients. At necropsy gross evidence of ulceration of the esophagus, just proximal to the cardia of the stomach, was found in six cases. Ulceration of the stomach was observed in six cases and was correlated with gross evidence of hemorrhage. One patient showed duodenal ulceration with evidence of hemorrhage on gross examination. Two patients in this series died as a direct result of their gastrointestinal ulceration and hemorrhage. One had massive ulceration with gastric perforation and generalized peritonitis. Our only outpatient death resulted from acute gastric distention which terminally was complicated by intussusception of the jejunum.

Another equally distressing complication was an unheralded ruptured appendix with peritonitis. Here the usual diagnostic armamentarium failed because of a comatose patient and absence of abdominal rigidity, which cannot occur in the presence of abdominal muscular paralysis.[14]

Gastrointestinal lesions in the acute bulbar poliomyelitic patient have been reported by Heyde and Robinson[16] as well as by Cook et al.[13] These authors have discussed the possible

etiology of these lesions and have pointed out the increased frequency of such lesions in the acute phase of the disease. Our own high incidence has demonstrated the prevalence of such complications in the chronic respirator patient.

Genitourinary System. The prevalence of renal calculi in our series, 80 per cent, is higher than the clinical incidence. (Table v.) Brady and Wilson[17] discuss this problem and the urologic surgical problems of the respirator patient. A number of evident factors influence this incidence. The respiratory alkalosis of so many of the patients, the tendency for urinary stasis, difficulties inherent in the acid-ash, low calcium diet, and the recurrent urinary infections and the difficulty in eradicating them in a paralyzed patient all serve to alert us to its import.[18,19] The associated urinary infection rate with complications needs no comment other than what has already been stated concerning its association with calculi, urinary stasis and the necessity for frequent catheterization in some patients. P. vulgaris and Ps. aeruginosa were the major offenders, with E. coli a frequent accompaniment

Why P. vulgaris and Ps. aeruginosa should be so prevalent in these infections is of interest and perhaps merits some comment.[21,23] The recognition of antibiotic-induced alterations in bowel bacterial flora may be the essential problem existing in these patients,[22] namely, that the microorganisms considered ubiquitous and of low pathogenic importance are now free to proliferate in body fluids uninhibited by many of their former normal antagonistic organisms destroyed by the action of antibiotics. The antibiotic-induced ascendancy of these organisms has resulted in greater interest in methods of controlling these new "antibiotic era" infections.

The average duration of life after onset of poliomyelitis for this group was 13.08 months. Two of the patients lived 3.5 and 4.5 years, respectively. If these two are excluded the average drops to 9.0 months. These figures are not very meaningful. At best they give only a slight indication of what may be expected with a severe respirator patient who survives the acute phase but continues in difficulties.

SUMMARY

Presented are case histories and tabulated summaries of the pertinent necropsy findings in fifteen deaths among the population of chronic bulbospinal poliomyelitic patients at Rancho Los Amigos Respiratory Center for Poliomyelitis. This represents the total mortality (2.05 per cent) from January 1, 1952, to July 15, 1954, at this Center. The deaths occurred, on the average, 13.08 months after the acute onset of bulbospinal poliomyelitis. The average age at death was 24.0 years, with extremes of forty years and six years of age. The sex distribution was twelve women and three men, although the census demonstrated an even sex distribution for the most part.

One-half (seven cases) of the deaths were directly attributable to some respiratory complication. Each body system is discussed in relation to complications.

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Evaluation of the "Positive" Urine Culture*

An Approach to the Differentiation of Significant Bacteria from Contaminants

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THE report of a "positive" culture on a urine specimen apparently collected aseptically may mean that clinically significant infection is present in either the urinary tract or the structures draining into it. However, this "positive" urine culture may also be the result of contamination during the collection or handling of the specimen and not associated with significant clinical disease, therefore the clinician and not the bacteriologist should make the final interpretation of laboratory findings. Rational therapy of urogenital infections, particularly when their course has been of a chronic nature, depends to a great extent upon accurate evaluation of urine cultures and their antibiotic susceptibility.[1] Furthermore, as Eisenberg and co-workers have stressed, "reliable, unprejudiced evaluation of antibiotic therapy depends on unequivocal establishment of the identity of the infecting flora."[2]

The usual method of determining the significance of any given bacterial strain is the demonstration of its constancy in the urine in the absence of specific therapy.[2,3] This method has the obvious disadvantage of requiring multiple pretherapy cultures, each taken with meticulous care. The delay this necessitates often cannot be afforded.

The difficulty in determining the significance of organisms found is compounded by antimicrobial therapy which may cause shifts in bacterial flora and the appearance of resistant bacterial strains or species.[1,4,5] Adequate evaluation as to whether these new strains are evidence of superinfection or are merely contaminants is important in further management. Multiple cultures before or during treatment, even if repeated daily, introduce further delay.

This problem of significant organisms has received more attention in connection with culture of other body fluids. Contamination is often more easily avoidable in such cultures, for example in cultures of blood, and the concentration as well as the type of bacteria recovered is more generally recognized to be of clinical importance.[6] The use of agar poured plates into which a known aliquot of blood is incorporated has been of great help in the evaluation of blood cultures. The necessity of performing quantitative bacterial counts on urine specimens was emphasized by Marple in 1940.[7] However, since that time no evidence of the use of such technics can be found in the literature.

A reiteration of the value of applying the poured plate technic to urine cultures and its correlation with the usual procedures applied in studying the urine from an individual with suspected infection forms the basis of this report.

MATERIALS AND METHODS

Specimens of urine were obtained by catheterization from patients with symptoms of urogenital infection or, as better termed, "obstructive-infectious

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uropathy."[8] Most of the specimens were collected by members of the urology service; a few were collected by nursing personnel. The urethral meatus and either the glans penis or vulva were cleaned with sterile cotton moistened with green soap. This washing was followed by cleansing with a 1:1000 aqueous zephiran® solution. The specimen of urine was drawn into a dry sterile test tube and sealed with a sterile cork stopper. Examination of the specimens was carried out within a two-hour period after collection.

The urine sample was divided aseptically into two aliquots. The first, 8 to 10 ml., was placed in a sterile conical centrifuge tube. After centrifugation at 1,800 r.p.m. for five to ten minutes the supernatant was decanted from the sediment. The sediment was studied as follows: (1) A portion of the sediment was removed with a sterile platinum wire loop, placed on a slide and stained with methylene blue. (2) A loopful of sediment was streaked onto a blood agar plate (when the methylene blue stain showed only cocci) or onto an eosin-methylene blue agar plate (pure bacilli or mixed cocci on the stained smear) and/or onto a blood agar plate containing 0.07 per cent sodium azide (mixed cocci and bacilli on the stained smear).[9] (3) In all instances thioglycolate broth was also inoculated with a loopful of the sediment. Single colony isolations were made after twenty-four hours' incubation of cultures at 37°C. On the basis of bacterial morphology (gross characteristics and microscopic examination of a Gram stain of representative colonies) and of biochemical reactions, pure strains were cultured on appropriate media and identified on the basis of the usual biochemical fermentations and serologic reactions. Cultures were incubated forty-eight hours before being discarded as negative. (4) To the remainder of the sediment was added a gentian violet-safranine stain described by Sternheimer and Malbin.[10] This was examined microscopically for formed elements.

The second portion of the initial specimen was kept in the tube into which it had been collected. After the sediment from the first aliquot was examined for bacteria, 1.0 ml. of the second portion was withdrawn with a sterile pipet to prepare a poured plate. The quantity of urine incorporated into the poured plate was based upon the number of bacteria seen in the sediment. If "none" or "rare" organisms were seen, 1.0 ml. of undiluted urine was used. If the sediment was "loaded" with bacteria, 0.1 ml. of original urine was diluted in 9.9 ml. of sterile distilled water (pH 7.0) and 0.1 ml. of this dilution (0.001 ml. of the original urine) was used; 1.0 ml. was pipetted into a sterile petri dish, to this dish was added 9.0 ml. of melted, cooled tryptic digest agar and mixing effected by swirling. When the contents of the pour plate solidified, it was incubated at 37°C. for eighteen to twenty-four hours and colonies counted under magnification in a Quebec colony counter. All counts were corrected to 1.0 ml. of urine. Using this technic, plates containing up to 1,000 bacterial colonies could be enumerated without much difficulty. Thus up to 1,000,000 colonies/ml. (10^6/ml.) could be quantitated. Concentrations greater than this were recorded merely as "loaded" or "innumerable." Representative colonies were isolated from the poured plate and Gram stain carried out to ascertain that the bacteria were of the same type as obtained from the cultures of the centrifuged sediment.

TABLE I
GENERAL BACTERIOLOGIC FINDINGS IN 250 CONSECUTIVE URINE CULTURES

Cultures	Number	Per cent	Pour Plates (bacteria/ml. urine)		
			Not Done	≤1,000	≥1,000
Positive cultures					
Single organism	169	67.6	16	44	109
Two organisms	31	12.4	3	9	19
Three organisms	10	4.0	2	2	6
Four organisms	1	0.4	0	0	1
Total positive cultures	211	84.4	21	55	135
Total negative cultures	39	15.6	..	..	...
Total cultures	250	....	..	..	...

The urine pH was estimated using nitrazine® paper after the aliquot for quantitative pour plate had been removed.

The level of significance of the various correlations was evaluated statistically, using two-by-two contingency tables and applying the method of chi square. The Yates correction for discontinuity was also applied. P values of 0.10 were considered questionably significant, 0.05 probably significant and 0.01 significant.

RESULTS AND COMMENTS

Two hundred fifty separate consecutive cultures of urine were studied and form the nucleus of this report. (Table I.) These cultures was obtained from 164 individuals, all of whom had clinical symptoms or signs of urogenital infection. Urine from some individuals was cultured several times. In no instance was more than one culture entered in the data for each distinct episode of infection. In this way no duplication of bacterial strains appears in the tables. Males predominated in this group (68 per cent). This percentage is comparable to the high proportion of males in other studies[11] in which Escherichia coli was isolated in low frequency. The majority of individuals in the present study had refractory infections or infections which had run a chronic course. These

are also factors which may favor a shift of their urinary tract bacterial ecology toward more resistant species.[4,12,13]

As noted in Table I 15.6 per cent of cultures showed no bacterial growth. Most of these cultures were obtained from persons with symptoms of nocturia, dysuria, urgency and frequency. Pyuria in this group was an uncommon finding. Other negative cultures of urine were obtained occasionally from persons with "extraluminal" genitourinary infections. These included prostatitis, epididymitis, orchitis and perinephric abscesses. Negative cultures were also obtained in persons with hematuria secondary to a neoplasm or calculary disease.

Cultures showed a mixed bacterial flora in 16.8 per cent of specimens examined. The majority of these contained only two species.

Poured plates were not obtained in twenty-one specimens. The number of specimens containing less than 1,000 colonies/ml. of urine was tabulated separately from those containing more than 1,000 colonies/ml. This separation value was based upon several criteria. The data showed a marked decrease in the number of specimens with greater than 1,000 organisms/ml. of urine. Furthermore, the distribution curve beyond 1,000/ml. approached a constant value. (Table II.) In addition there were marked shifts in ecology at approximately this level of bacteriuria. (Table III.) Strains present at concentrations less than 1,000 colonies/ml. tended to be inconstant. Similar numbers and types of bacteria were isolated from the urine of females when "clean-voided" specimens were studied.[14] It should be emphasized that this figure of 1,000 colonies/ml. is not absolute but rather an order of magnitude. Its use in determining treatment, particularly in borderline instances, will require the application of clinical judgment.

When this bacteriologic criterion was used on our data, fifty-five of 190 (29 per cent) cultures on which quantitative counts were performed had seemingly "insignificant" numbers of bacteria. Although a few of this group had either "extraluminal" infections or were almost free of infection after successful therapy when first studied, the majority of these cultures contained bacteria which must be considered as contaminants.

Observations regarding bacterial strains and other features of the urinary sediment were made only on cultures demonstrating significant bacteriuria, except where specifically noted to the contrary.

TABLE II
DISTRIBUTION OF CULTURES CONTAINING SMALL NUMBERS OF BACTERIA

Bacterial Count (colonies/ml.)	No. of Cultures
0	10
1–100	21
101–500	13
501–1,000	9
1,001–2,000	3
2,001–3,000	2
3,001–4,000	3
4,001–5,000	1
5,001–6,000	4
6,001–7,000	0
7,001–8,000	4
8,001–9,000	0
9,001–10,000	2

TABLE III
CORRELATION BETWEEN BACTERIAL COUNTS AND SPECIES ISOLATED*

Bacterial Species	Bacterial Count (colonies/ml.)			
	0–1,000		≥1,000	
	No.	%	No.	%
Staphylococci	16		5	
Coagulase-negative	14	36.0	3	2.9†
Coagulase-positive	2	5.1	2	1.9
Enterococci	4	10.2	9	8.7
Proteus species	6	15.4	20	19.4
Pseudomonas species	0	.....	5	4.9
Escherichia coli	3	7.7	16	15.5
Aerobacter aerogenes	2	5.1	34	33.0†
Klebsiella pneumoniae	1	2.6	8	7.8
Paracolon	0	.....	1	1.0
Diphtheroids	1	2.6	1	1.0
Escherichia intermedium	0	.....	2	1.9
Alcaligenes faecalis	4	10.2	2	1.9†
Miscellaneous (α-streptococci and candida)	2	5.1	0	.....
Total	39	100.0	103	99.9

* Based upon cultures with single organisms.
† Differences significant (P = 0.10 or less).

The relative preponderance of the type of bacterial species isolated changed with the degree of bacteriuria. (Table III.) Coagulase-negative staphylococci, of which the majority were non-hemolytic and non-pigmented (albus), and Alcaligenes faecalis were species more commonly found in cultures with less than 1,000 colonies/ml. Most of these strains were considered to be contaminants; in some instances, however, they appeared to be etiologic agents. Aerobacter aerogenes and probably E. coli occurred more frequently in larger numbers than in smaller numbers. These species were considered to be infrequent contaminants. It should be emphasized that the mere presence or absence of any given microorganism is not an adequate basis for ruling it in or out as a cause of clinical urogenital disease.

Microscopic examination of the urine sediment for formed elements, particularly leukocytes, erythrocytes and casts, as well as for stainable bacteria, is of great importance in the diagnosis of infection.[15,16] The correlation between bacterial counts and microscopic examination of the centrifuged urinary sediment is tabulated in Table IV. Two important conclusions seemed apparent in this analysis. First, as has long been recognized,[11,15,17] the absence of pyuria in single or multiple urine specimens did not negate the diagnosis of urinary tract infection. Leukocytes were not demonstrable in thirteen of ninety-one specimens with large numbers of bacteria (>10,000/ml.). Neither did the presence of pyuria always imply that a pyogenic urinary tract infection was present. Second, when bacteria were seen in moderate numbers (one to two bacteria/oil field) on smear, they could be cultured in significant numbers (>1,000/ml.) Approximately 5,000 to 10,000 viable organisms/ml. had to be present before they could be seen on stained smears of the centrifuged sediment. In specimens with sterile cultures no bacteria were seen, while in those specimens with >10,000 bacteria/ml. bacteria were apparent in all instances.

The effect of the various species of bacteria upon the urine sediment is tabulated in Table V. Striking variations from the expected findings were noted only with infections due to proteus species, i.e., in six of twenty specimens the pH

TABLE IV
CORRELATION BETWEEN BACTERIAL COUNTS AND SEDIMENT*

No. "Positive" Cultures Studied	No. Organisms/ml. on Pour Plate	Bacteria in Stained Sediment				WBC/HPF in Sediment		
		0	Rare	1-2+	3-4+	0	1-10	≥10
10	0	10	0	0	0	9	0	1
34	1-1,000	28	4	2†	0	29	4	1
18	1,000-10,000	13	2	3	0	15	2	1
91	≥10,000	0	0	26	65	13	41	37
Total: 153								

* Based upon cultures with a single organism.
† Both contained cocci.

TABLE V
CORRELATION BETWEEN BACTERIAL SPECIES, URINE pH AND PYURIA*

Bacterial Species	No. of Strains Studied	Urine pH			WBC/HPF		
		4.5	5.0-6.5	7.0	0	1-10	>10
Staphylococci	5						
Coagulase-negative	3	0	3	0	0	1	2
Coagulase-positive	2	0	2	0	0	0	2
Enterococci	9	0	9	0	3	3	3
Proteus species	20	1	13	6†	8†	9	3
Pseudomonas species	5	0	5	0	1	4	0
Escherichia coli	16	1	14	1	2	6	8
Aerobacter aerogenes	34	2	31	1	4	16	14
Klebsiella pneumoniae	8	0	8	0	4	0	4
Paracolon	1	0	0	1	0	1	0
Diphtheroids	1	0	1	0	0	1	0
Escherichia intermedium	2	0	1	1	2	0	0
Alcaligenes faecalis	2	0	2	0	2	0	0
Total	103	4	89	10	26	41	36

* Based upon cultures with significant numbers of single bacterial species.
† Differences significant (P — 0.10 or less).

TABLE VI
CORRELATION BETWEEN PURE CULTURES AND MIXED CULTURES

Bacterial Species	Pure Cultures		Mixed Cultures	
	No.	%	No.	%
Staphylococci				
Coagulase-negative	3	3.0	4	13.3*
Coagulase-positive	2	2.0	2	6.7
Enterococci	9	8.9	3	10.0
Proteus species	20	19.8	6	20.0
Pseudomonas species	5	5.0	2	6.7
Escherichia coli	16	15.8	3	10.0
Aerobacter aerogenes	34	33.6	5	16.7*
Klebsiella pneumoniae	8	8.0	2	6.7
Escherichia intermedium	2	2.0	2	6.7
Alcaligenes faecalis	2	2.0	1	3.3
Total	101	100.1	30	100.1

* Differences questionably significant (P — 0.10).

was 7.0 or greater (P = 0.10 or questionably significant). Rhoads and co-workers have commented upon the absence of excessive pyuria associated with infections due to Streptococcus faecalis.[1] In most urinary tract infections, except for approximately one-third of those due to proteus species, the pH of freshly obtained specimens remained within normal limits. The absence of pyuria bore little relationship to the causative bacteria, again with the possible exception of strains of proteus.

The bacterial species isolated from mixed cultures had a frequency of occurrence similar to that of pure cultures. (Table VI.) The presence of two bacterial species in significant number was based upon finding more than 1,000 colonies/ml. of urine, and upon the identification of both strains on the initial examination of the stained sediment. In this very small series there were two variations from the expected incidence, based on pure cultures. Coagulase-negative staphylococci were more frequent than expected (P = less than 0.10) and A. aerogenes less common (P = 0.10).

The application of this concept and technic to clinical problems is best illustrated by a few selected representative problems.

CASE REPORTS

CASE I. (No. 9A44.) A. R., a sixty-three year old white housewife, had a three-year history of urinary frequency. Recurrent episodes of "cystitis" followed upper respiratory infections since 1953. Intravenous urograms suggested chronic pyelonephritis. Urethral stenosis of moderate degree was present. Bacteriologic summary was as follows:

Date (1954)	Pour Plate (colonies/ml.)	Culture	Culture Site	Therapy and Comments
1/18	Innumerable	E. coli	Bladder	WIN 5094-2 started
1/22	0	Proteus species	.....	WIN 5094–2 stopped; chloramphenicol started
1/25	250	Proteus species	.....	.....
2/2	.....	.....	.....	Chloramphenicol stopped
2/10	2	Proteus species	.....	Asymptomatic; also asymptomatic in March and June 1954

Comment. This is an example of cystitis due to E. coli which responded well to antibiotic therapy. Repeat cultures contained insignificant numbers of organisms. Without a quantitative method of study these findings could have been interpreted as superinfection with a strain of proteus. While they might have been potential etiologic agents for superinfection, they were of no consequence.

CASE II. (No. 4G105.) H. W., a seventy-one year old man, had a right lower lobe lobectomy on October 19, 1953, for an epidermoid carcinoma of the lung. Postoperatively, following continuous catheter drainage symptoms of frequency, urgency and nocturia developed. Therapy consisted of penicillin, streptomycin, chloretetracycline, oxytetracycline, several sulfonamides, mandelamine,® chloramphenicol and WIN 5094-2. A perineal-prostatectomy was performed on April 5, 1954, because of slight median lobe enlargement and a residual urine volume of approximately 125 ml. Pathologic sections demonstrated adenomatous prostatic hyperplasia and focal chronic active prostatitis. Postoperatively he became asymptomatic without antibiotic therapy. Bacteriologic summary was as follows:

Date	Pour Plate (colonies/ml.)	Culture	Culture Site	Therapy and Comments
11/28/53	Innumerable	A. aerogenes	Bladder	Multiple antibiotics
1/11/54	Innumerable	A. aerogenes	.....	.....
2/8/54	Innumerable	A. aerogenes	.....	.....
2/27/54	Innumerable	A. aerogenes	.....	.....
3/25/54	Innumerable	A. aerogenes	.....	.....
3/29/54	Innumerable	A. aerogenes	.....	.....
4/1/54	Innumerable	Paracolobacterium aeruginoides	.....	.....
4/5/54	.....	.....	.....	Perineal prostatectomy
6/7/54	124	Staphylococcus, coagulase-negative	.....	Asymptomatic

Comment. This was an example of the ineffectiveness of antimicrobial agents alone in controlling "obstructive-infectious" uropathy and of the prompt response which followed correction of a mechanical lesion.

CASE III. (No. 5G161.) E. S., a sixty-one year old white housewife, had diabetes mellitus of twelve years' duration. For the past year she had had intermittent passage of "brownish red" urine associated with an elevation in temperature to about 101°F., usually of one to two days' duration. Upon physical examination the left kidney was palpable. On cystoscopic examination leukocytes were seen in the urine obtained from the left ureter. A retrograde pyelogram revealed an irregularity of the lower calyx on the left. Bacteriologic summary was as follows:

Date	Pour Plate (colonies/ml.)	Culture	Culture Site	Therapy and Comments
12/29/53	4	Klebsiella pneumoniae	Right ureter	None
	2,000,000	Klebsiella pneumoniae	Left ureter	....
	Innumerable	Klebsiella pneumoniae	Bladder	....

Comment. Routine cultures of the urine obtained at cystoscopy would have probably suggested bilateral infection in spite of the clinical opinion that this was a left pyelonephritis. The pour plate demonstrated clearly that a significant number of bacteria were being shed only from the left kidney.

CASE IV. (No. 9G67.) M. M., a seventy-six year old white laborer, had a history of nocturia, dysuria and progressive decrease in the caliber of his urinary stream. Definite prostatic enlargement was present. Bacteriologic summary was as follows:

Date	Pour Plate (colonies/ml.)	Culture	Culture Site	Therapy and Comments
6/4/54	92	A. faecalis	Right ureter	None
	74	A. faecalis	Left ureter	....
	Innumerable	A. faecalis	Bladder	....
	Few	Staphylococcus, coagulase-negative	.....	....

Comment. Routine cultures would probably be interpreted as indicating bilateral pyelonephritis and cystitis. The quantitative counts confirmed the more accurate clinical impression of cystitis in the absence of pyelonephritis.

SUMMARY

1. A method for quantitative bacterial counts, using the agar pour plate technic as a part of the procedure for urine cultures, is described. This procedure offers advantages in the rational management of infections of the urinary tract. It also permits a more reliable evaluation of therapy.

2. A degree of bacteriuria approximating 1,000 viable organisms/ml. of urine is required for the certain diagnosis of infection. Extraluminal infections or subsiding urogenital infections may be associated with a lower urinary concentration of bacteria.

3. Quantitative studies have shown that the presence of a moderate number of bacteria on a stained smear is highly suggestive of significant bacteriuria. Pyuria during infection is variable and fails to correlate closely with clinically significant bacteriuria.

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Hodgkin's Disease and Immunity*

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The symptom-complex, Hodgkin's disease, ranges the spectrum of disease manifestation from benign granuloma to malignant sarcoma. At this time diagnosis of this disorder can be no more certain than description allows, nor is there a specific test for diagnosis. Its etiology is as yet unknown. The disease has not been unquestionably transmitted to experimental animals. The encyclopedic review by Hoster et al.[1] details the attempts to uncover the nature of this disease. Hodgkin's disease has been aptly termed by the French, "maladie de frontière."

Our interest in Hodgkin's disease has been to delineate the skin anergy associated with it. The association of Hodgkin's disease with a reduced or absent tuberculin reaction is familiar.[2] Further observations disclosed that this anergy to tuberculin was not specific but that patients with Hodgkin's disease reacted similarly to a group of delayed reacting antigens; namely, mumps, Trichophyton gypseum, Candida albicans, as well as to purified protein derivative of tuberculin.[3] Dubin[4] noted the poverty of the immunologic mechanism in patients with Hodgkin's disease, specifically the lower incidence of positive serologic reactions for syphilis; the inability to produce antibodies against coexistent brucellosis; and the failure of a patient to respond to typhoid vaccine by production of antibodies.

In the past, Hodgkin's disease has been associated with tuberculosis and was once thought by Sternberg to be a variant of this latter disease. A review by Parker and Jackson[5] in 1932 of 400 general autopsies and 151 autopsies in lymphomas and leukemias demonstrated a significantly higher incidence of healed and active tuberculosis in Hodgkin's disease as contrasted with its occurrence in general autopsies, terminal carcinomas and the leukemias. Ewing has said, "In New York City tuberculosis follows Hodgkin's disease like a shadow."

Similarly, Hodgkin's disease was associated with brucellosis in fourteen patients described by Wise and Poston.[6] The organisms were cultured from either the blood or the affected lymph nodes, or both. These studies, although carried out in an endemic area for brucellosis, suggest a more than chance relationship.

The lack of general agreement concerning the association of these indolent infections and Hodgkin's disease is a result of disparity in sampling. These associations seem to have been more frequent in advanced or terminally ill patients with this disease.

Recently, a less equivocal relation was made apparent between Hodgkin's disease and infection with Cryptococcus neoformans. A review by Gendel et al.[7] lists 165 reported cases of infection with C. neoformans, in fourteen of which Hodgkin's disease was also present. A higher incidence is reported by Zimmerman and Rappaport[8] at the Armed Forces Institute of Pathology; eleven of sixty patients with C. neoformans were observed to have Hodgkin's disease.

These clinical data suggested a defect in immunity and offered a clue to the association of Hodgkin's disease with the indolent infections, the antigens of which induce delayed type skin reactions. The following studies were undertaken to elucidate the immunologic response in Hodgkin's disease: (1) Extension of observations on cutaneous anergy; (2) assays of the development of complement fixing antibodies to mumps virus; (3) the passive transfer of sensitivity; (4) determination of serum complement levels.

METHODS AND MATERIALS

Forty-three patients with Hodgkin's disease were studied. The diagnosis in all was established by lymph node biopsy. Only ambulatory patients with Hodgkin's disease were included in this study because of the reputed attenuated cutaneous response of cachectic

* From the Medical and Laboratory Services, Veterans Administration Hospital, Brooklyn, New York.

TABLE I

CUTANEOUS RESPONSE TO ANTIGENS							
GROUP	NO.	HISTAMINE	MUMPS CONTROL	MUMPS	CANDIDA ALBICANS	TRICHOPHYTON GYPSEUM	PPD
Controls	79	100%	0.6%	90±3.4%	92±3.1%	68±5.2%	71±5.1%
Hodgkin's	43	100%	0%	14±5.3%	19±6.0%	16±5.3%	23±6.5%
"P" values				<.0001	<.0001	<.0001	<.0001

Numerical values following ± sign represent S. E.

and severely ill patients. Seventy-nine subjects selected for the control group were without evidence of Hodgkin's disease; they were without fever and had normal white cell and red cell counts. Some were patients with such diverse diagnoses as duodenal ulcer, diabetes mellitus, cirrhosis of the liver and inactive rheumatic heart disease. The remainder were physicians and nurses.

Purified protein derivative (P.P.D.) of intermediate strength (0.001 mg.) was used to determine the reaction of each individual to tuberculin. Simultaneously, tests were made with histamine phosphate (0.05 mg.), 1:1000 dilution, and four unrelated antigens, which also induce delayed responses. These were mumps virus, C. albicans, T. gypseum and normal allantoic fluid of chick embryo as a control for the mumps virus antigen.* Extracts of C. albicans and T. gypseum were used in a dilution of 1:150. The antigens were injected in 0.1 ml. doses intracutaneously, and the reactions were read after forty-eight hours. The greatest diameter of the area of edema (3 mm. or larger) was measured. Erythema was not recorded because of the difficulty of accurate estimation in pigmented persons. Separate syringes and needles for each antigen were employed to avoid mixed reactions.

Immunization with mumps virus vaccine followed the preliminary skin testing. Two doses of 0.5 ml. each of mumps vaccine were given subcutaneously two weeks apart. Pre-, mid- and postimmunization samples of blood for mumps complement-fixing antibody titers were drawn; the final sample being taken four weeks after the first immunization. Specimens of blood for complement assay were conveniently taken simultaneously. They were immediately placed in iced tubes, allowed to clot, and centrifuged at 0°c. The serum obtained was then sealed in chemically clean and sterile glass tubes and stored at −20°c. The three separate specimens of serum on each individual were then assayed.

* The antigens of C. albicans and T. gypseum were obtained from Hollister-Stier Lab., Philadelphia. P.P.D. was purchased from Parke Davis & Co., Detroit, Mich. The mumps skin testing antigen and complement fixing antigen were kindly supplied by Dr. Victor Cabasso, Section of Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamide Company, Pearl River, New York.

Mumps complement-fixing antibody titers were determined according to the method of Bengston.[9] The complement fixing antigen routinely used for the testing of all sera was a fivefold concentrated infected allantoic fluid which contained 0.1 per cent formalin.*

The amount of serum necessary to produce 50 per cent hemolysis was determined spectophotometrically and the results were calculated from the Van Krogh alternation formula, $\log x = \log K + \frac{1}{n} \log \frac{y}{1-y}$, (Kabat and Mayer).[10]

Passive transfer studies (Prausnitz-Küstner reactions) were conducted by the injection intracutaneously on one arm of 0.1 ml. of Seitz filtered serum from a person sensitive to ragweed. This area and a control site on the opposite arm of the individual were both challenged forty-eight hours later with 1:1000 ragweed extract. The greatest diameter of the wheal was recorded twenty minutes later.

RESULTS

In the control group there were no significant differences in diameter or frequency of positive reactions between white and Negro subjects, between normal persons and those with disease, or between those of different ages.

Cutaneous Reactions to Delayed Response Antigens and Histamine (*Table* 1). *Histamine.* Both the control patients and those with Hodgkin's disease reacted with the development of wheals. These were measured twenty minutes after injection. The response was identical in the two groups.

Mumps control: There was one positive reactor to the normal allantoic fluid of chick embryo of the 132 people tested.

Mumps antigen: The control group had 90 per cent positive reactors as compared with 14 per cent among the patients with Hodgkin's disease. The group difference was apparent in the average diameter of the wheal as well as the frequency.

Candida albicans: Positive reactors were found in 92 per cent of the control group in contrast to 19 per cent of the group with Hodgkin's dis-

ease. Here too there was a significant difference in the average size of reactions in the two groups.

Trichophyton gypseum: There were positive reactors in 68 per cent of the control group and 16 per cent of the patients with Hodgkin's disease. The patients with Hodgkin's disease gave a smaller response.

TABLE II

IMMUNOLOGIC RESPONSE TO MUMPS VACCINATION					
GROUP	NO.	GEOMETRIC MEAN ANTIBODY LEVEL			
		PRE TITER		*POST TITER*	
		SOLUBLE	VIRAL	SOLUBLE	VIRAL
Normal	14	1:69	1:62	1:64	1:67.3
Hodgkin's	12	1:11.3	1:56	1:76	1:95.7

Tuberculin: Similarly, 71 per cent of the control group gave positive skin reactions to P.P.D. Of the patients with Hodgkin's disease, 23 per cent had positive reactions. This accords with the observations of Steiner[2] and others.[1]

Immunologic Response to Mumps Vaccination. The pre- and postinnoculation titers of twelve patients with Hodgkin's disease were compared with those of fourteen normal subjects. Table II illustrates the similarity of the two groups indicating that the group with Hodgkin's disease responded with the appearance of circulating complement fixing antibody akin to normals.

Passive Transfer of Sensitivity to Ragweed Extract. The fourteen patients with Hodgkin's disease reacted in the same manner as normal persons. In all immediate wheals and erythema developed in the same fashion as with histamine. These studies indicate that patients with Hodgkin's disease when passively sensitized are capable of normal cutaneous responses to antigens producing immediate reactions.

Complement Levels. Although the group with Hodgkin's disease showed a higher mean level of serum complement than the normal group, no significant changes were noted during the course of immunization. (Table III.) Arithmetic means were taken of the three specimens on each individual — the mean of the group with Hodgkin's disease (C_{H50}) was 3.07 ml. $\times 10^{-3}$ of serum necessary to produce 50 per cent hemolysis of sensitized sheep red blood cells as compared with 4.40 $\times 10^{-3}$ ml. of serum in the normal group. Our results are in accord with those of Fischel[12] and Pitner.[13]

DISCUSSION

Although the precise mechanism of response to delayed reacting antigens is obscure, it is suggested that the skin anergy observed in patients with Hodgkin's disease is a manifestation of depression of tissue immunity. It appears that the susceptibility to indolent infections of patients with Hodgkin's disease is related to this phenomenon. In this group of patients studied, two of forty-three have eventually succumbed, respectively, to meningitis (C. neoformans) and to aspergillosis of the lungs.

TABLE III

SERUM COMPLEMENT LEVELS AS C'_{H50}		
GROUP	NO.	LEVELS
Controls	13	$4.4 \pm .056 \times 10^{-3}$ ml
Hodgkin's	13	$3.1 \pm .011 \times 10^{-3}$ ml
"P" values		$< .0001$

The diverse symptomatology of patients with Hodgkin's disease, including fever, headache, glandular swelling, weight loss, cough, paralysis or paresis, may conceal an underlying infection with tuberculosis, brucellosis, C. neoformans or other indolent infection. Before initiation of cortisone therapy it is advisable to rule out these additional possibilities to avoid rapid spread and overwhelming infection.

The remarkably low incidence of tuberculosis in leukemias has been mentioned.[5] This observation refutes the explanation attributing the spread of tuberculosis in patients with Hodgkin's disease to destruction of foci containing tubercule bacilli by radiation. If this were true, the same incidence of tuberculosis would be expected in cases of both Hodgkin's disease and leukemia.

It is surprising that Hodgkin's disease, which is characterized by widespread involvement of the reticuloendothelial system, gave no evidence of interference with the production of humoral antibodies, which are thought to be produced by this system. Our data are in agreement with those of Hoffman and Rottino[14] who studied the antibody response of thirteen patients with Hodgkin's disease immunized with a standard dose of typhoid-paratyphoid vaccine. Using a group of student nurses as controls, they were unable to demonstrate any significant difference in antibody response in the two groups. Larson

and Tomlinson,[15] using the technic of Heidelberger, observed the diminished production of antibodies to types 2 and 3 pneumococcus polysaccharide in three of six patients with Hodgkin's disease. Three produced antibodies normally. However, Geller[16] described significant antibody formation in only nine of sixteen normal subjects using this method, and type 1 pneumococcus polysaccharide. Of the four untreated patients with Hodgkin's disease he studied, one produced antibodies in normal quantity, two did not and one had a significant level initially. Of five patients with Hodgkin's disease who were treated, one produced significant amounts of antibody, three did not and data were incomplete in one.

The humoral antibody response of the Hodgkin's patients paralleled that of the normals. Thirteen of the fourteen normals had positive skin test to mumps before immunization, indicating prior exposure to the antigen. In the group with Hodgkin's disease only one patient had a positive skin test prior to immunization. Nevertheless, the geometric mean rise in antibody titer of the patients with this disease duplicated that of the controls which is indicative of an anamnestic response. It is assumed, therefore, that the patients with Hodgkin's disease had lost skin reactivity. This was actually observed in one patient who, nine months after giving a strongly positive reaction to the skin test (13 mm. wheal), was anergic to the mumps skin-testing antigen. According to the statistical data presented by Wesselhoeft,[17] 80 per cent of the urban population will have contracted mumps by the age of seventeen. Enders et al.[18] consider the skin test a reliable and convenient method of revealing most of the resistant individuals and somewhat more sensitive than the complement fixation test as an index of the immune state. It is unlikely that there should be one positive skin reactor in the fourteen patients with Hodgkin's disease who produced normal amounts of circulating antibody. These data on skin testing with mumps antigen followed by immunization therefore suggest that the patients with Hodgkin's disease lost their reactivity to mumps skin testing antigen but retained the ability to react to immunization with a rise in circulating antibodies consistent with an anamnestic response.

The capacity of the tissue of patients with Hodgkin's disease to react was demonstrated by successful passive transfer of ragweed antibodies from a normal subject to a subject in the Hodgkin's group. This was further supported by normal reactions to histamine by patients with Hodgkin's disease.

It is presumed that the diminished skin sensitivity to a variety of unrelated delayed-reacting antigens is a result of a defect in production or transport of the cellular antibodies concerned in these reactions.

Complement levels in patients with Hodgkin's disease were greater than normal, indicating that lack of total complement is not the explanation for the discrepancy in cutaneous response. However, information by Pillemer et al.[19] shows that another component of serum may be an important factor in natural immunity. This protein, tentatively named properdin, acts only in conjunction with C′3 of complement and magnesium ion and participates in such diverse activities as the destruction of bacteria, the neutralization of viruses, and the lysis of certain red cells. It does not necessarily parallel the levels of C′3 or total complement. A deficiency of properdin might still be found.

These observations demonstrate that the immunologic defect in Hodgkin's disease is a limited one, unrelated to the production of circulating antibody, complement levels or capability of the tissue to respond.

In our studies there was no correlation between the magnitude and incidence of skin reactivity in the patients with Hodgkin's disease to the severity and duration of the disease, or treatment with cortisone, radiation or nitrogen mustard. Six of the twelve patients with Hodgkin's disease in the antibody study were receiving cortisone in doses up to 150 mg. a day during the course of their immunization. Four additional patients received nitrogen mustard and seven, radiation. Two had all three modalities. The response of these patients could not be distinguished from either the controls or other patients with Hodgkin's disease.

Fischel[20] discusses the potential implications of current investigation of the effects of cortisone therapy in relation to antibody production. He concluded that the clinical efficacy of cortisone probably bears little relation to antibody inhibition except in certain specific disorders such as acquired hemolytic anemia in which clinical improvement usually coincides with disappearance of abnormal circulating antibodies from the blood. It was emphasized that, although it has been clearly demonstrated that antibody syn-

thesis is depressed by cortisone, the depression is small in extent and occurs only when extraordinarily large quantities of the hormones are employed. That the antibody effect is probably not of major importance in the therapy of allergic states is evident in patients with asthma or hayfever whose symptoms may be adequately controlled by cortisone but in whom sufficient reagin or antibody is present to give satisfactory skin reactions.

This was supported by evidence in our studies of successful passive transfer of sensitivity and maintenance of reactivity to histamine in spite of treatment with cortisone, nitrogen mustard and radiation.

There is an interesting parallel between the response of patients with Hodgkin's disease and patients with sarcoidosis to these immunologic tests as well as the recognized susceptibility of both groups to tuberculosis. Friou[21] observed the diminished response to delayed-reacting antigens in sarcoidosis. Sones and Israel[22] extended these observations and noted successful passive transfer of sensitivity to ragweed as well as failure of development of usual skin sensitivity after pertussis agglutinogen immunization, although circulating antibodies appeared in normal titers. It seems likely that the collagen diseases which are associated with increased tissue reactivity may be mutually exclusive with both Hodgkin's disease and sarcoidosis. In addition, preliminary observations indicate that allied diseases to Hodgkin's such as reticulum cell sarcoma, lymphosarcoma, lymphoblastoma will fall in the latter category (not leukemias, however).

SUMMARY

1. Forty-three patients with Hodgkin's disease have been studied with a variety of skin tests and immunologic procedures.

2. Patients with Hodgkin's disease were found to react significantly less often than controls by skin testing to tuberculin, mumps virus, C. albicans and T. gypseum.

3. In spite of negative skin tests to mumps antigen, following immunization with mumps vaccine, circulating antibodies appeared in titers consistent with an anamnestic response.

4. Normal responses were observed to passive transfer of ragweed sensitivity and to intradermal histamine injection.

5. Complement levels in patients with Hodgkin's disease were higher than normal.

6. These observations demonstrate that the immunologic defect in Hodgkin's disease is a limited one. It is suggested that the defect involves the production or transport of antibodies associated with delayed skin reactions.

7. Cortisone, nitrogen mustard and radiation, as customarily used in patients with Hodgkin's disease, were without effect on the passive transfer of ragweed sensitivity or the production of antibodies to mumps virus.

8. It is suggested that this defect in immunity, loss of reactivity to delayed-reacting antigens, offers an explanation for the association of Hodgkin's disease with the indolent infections.

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Treatment of Bromide Intoxication with Mercurial Diuretics*

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ALTHOUGH the first clinical description of bromide intoxication appeared in 1850,[1] it remained for Wuth in 1927 to bring about a clearer understanding of this clinical entity. He devised a simple method for determination of bromide blood levels and was the first to recommend sodium chloride administration in the treatment of bromism.[2]

The aim in treatment of bromide intoxication is to promote quick elimination of the bromide ion from the body. Until the present time, administration of sodium chloride in the amount of 4 to 8 gm. per day, orally or parenterally, has been the standard therapeutic regimen in the treatment of this entity. The rationale of such therapy is based on the known fact that the chloride ion displaces bromide ion when bromides are withheld and chlorides are made available to the body tissues. The liberated bromide is then excreted in the urine.

Unfortunately, the sodium chloride treatment of bromide intoxication cannot be considered satisfactory. The bromides are eliminated slowly, making the period of recovery prolonged. The effect of sodium chloride administration on the rate of renal elimination of bromides is shown in a graph by Goodman and Gilman,[3] which indicates that a period of approximately twenty-three days was required in the instance illustrated for the serum bromide level to fall from 350 mg. per cent to 130 mg. per cent. Various subsequent clinical observations have confirmed these findings. Cross[4] reports that the sodium chloride therapeutic regimen may require from three to six weeks for the symptoms of the intoxication to disappear. Likewise, Wohl and Robertson[5] found an average of twenty-three days necessary and Perkins'[6] patients required an average of seventeen days for complete disappearance of symptoms after the salt therapy.

A review of the literature reveals a sparsity of investigations designed to improve the therapeutic results in this entity. This fact has been due at least in part to the conclusion of some clinicians that too rapid mobilization of bromides from the tissues may temporarily increase the serum bromide level and thus aggravate clinical symptoms. However, such a therapeutic paradox remains controversial, and there is little proof to justify this belief.

Earlier modification of the "salt" therapy produced some promising results. Wohl and Robertson treated seven patients with a combination of desoxycorticosterone and sodium chloride. The average time for disappearance of symptoms was nine and three-quarter days and the blood bromide level was reduced by 50 per cent in four to eight days as compared with eight and a half to fourteen and a half days for patients treated with sodium chloride alone. One patient was treated with desoxycorticosterone alone without beneficial effect.[5] Bondurant and Campbell[7] reported good results with adrenal-cortical extract therapy in seven cases of bromide intoxication.

Harris and Derian[8] administered 750 mg. of niacinamide daily to six patients; four received no sodium chloride but two were given 6 gm. per day in addition to the vitamin therapy. The average time required for the symptoms to disappear after this therapy was approximately five days. In these patients there was a remission of symptoms even though a high blood bromide level was maintained. The addition of sodium chloride to this regimen resulted in a gradual decrease in the blood bromide level.[8] Recently Cornbleet[9] has shown that ammonium chloride produces a more rapid decline of blood bromide levels than does sodium chloride administration.

Bromide ions are eliminated very slowly from the body. After a single dose of 2 gm. the urine

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shows the presence of bromide for two months. This indicates that patients taking even small doses will in time accumulate enough bromide in the tissues to produce toxic symptoms.

Apparently clinicians have failed to realize the significance of the work of Palmer and Clarke[10] and of Bodansky and Modell[11] who demonstrated the fallacy of the theory that the kidney does not differentiate between the excretion of the chloride and bromide ions. These investigators have shown in dogs that the kidney excretes chloride preferentially over bromide; in other words, the urinary bromide-halide ratio is smaller than the plasma bromide-halide ratio, therefore, in the formula

$$K = \frac{\text{Br (urine)} : \text{Total halide (urine)}}{\text{Br (serum)} : \text{Total halide (serum)}},$$

K has a value below 1. In their experiments, the value of K was strikingly constant, around 0.4, after bromide administration and during an average but constant chloride intake. This differential excretion of chloride and bromide permits the accumulation of bromides in the tissues even in patients receiving small doses of the drug.

Furthermore, these investigators have demonstrated that the ratio of bromide excretion depends on both the total halide excreted in the urine and the concentration of total halide in the plasma. Therefore, the administration of either bromide or chloride increases the bromide excretion absolutely and proportionately to the chloride. These results explain the beneficial effect of large amounts of chloride administration on the renal elimination of bromide. The administration of chlorides, then, will not only increase the total halide excretion but also increase the bromide fraction in the urine.

Investigation of additional factors that might increase urinary bromide excretion have shown that (1) the administration of large amounts of water does not appreciably increase the excretion of halide although the urine output is increased; (2) the administration of urea increases the urinary volume without affecting bromide excretion; (3) the injection of theophylline increases the total halide and bromide excretion; (4) injection of salyrgan® into the dog produces a striking increase in excretion of total halide, consisting of increase in both chloride and bromide, and a rise in the bromide-halide ratio in the urine.[10,11]

It has been postulated that the preferential excretion of chloride results from the known fact that the bromide ion moves across membranes slightly more rapidly than the chloride ion and therefore is more quickly reabsorbed in the tubules from the glomerular filtrate than the chloride ions.[12]

TABLE 1
BROMIDE INTOXICATION TREATED WITH MERCUHYDRIN
Case 1 (C. W.)

Day	Serum Bromide Level (mg./100 cc.)	Urine			Mercuhydrin Administered
		24-Hour Volume (cc.)	Bromide Level (mg./100 cc.)	Bromide Excreted (gm.)	
1	186	835	142 (?)	1.18	..
2	186	460	38	0.17	..
3	...	2,650	83	2.20	x
4	...	2,100	23	0.48	..
5	175	3,150	26	0.82	..
6	163	3,140	75	2.35	x
7	149	2,780	40	1.11	..

If the preferential excretion of chloride ultimately depends upon the extent of preferential reabsorption of bromide in the tubules, one would expect the mercurial diuretics to reverse this situation, inasmuch as this diuretic has a primary effect on the tubular epithelium, rendering reabsorption of water and salts difficult. On this premise, we decided to investigate the value of mercurial diuretics in the treatment of bromism.

EXPERIMENTAL STUDIES AND CASE REPORTS

Following a preliminary investigation on three patients reported elsewhere,[13] our studies have been extended to eleven patients hospitalized with chronic bromide intoxication. In this study we attempted to obtain answers to the following questions:

1. Does mercuhydrin® increase urinary bromide excretion?*

CASE 1. On the first day of observation this patient (C. W.) had a blood bromide level of 186 mg. per cent. (Table 1.) Subsequent blood bromide levels were determined daily and twenty-four-hour urine specimens were collected. The urine specimens were measured and examined for the bromide concentration per cubic centimeter and total bromide content expressed in grams. In the control period of two days

* Manufactured by Lakeside Laboratories, Inc., Milwaukee, Wisconsin.

TABLE II

BROMIDE INTOXICATION TREATED WITH SODIUM CHLORIDE AND MERCUHYDRIN

Case II (H. H.)

Day	Serum Bromide Level (mg./100 cc.)	Urine			NaCl Administered	Mercuhydrin Administered
		24-Hour Volume (cc.)	Bromide Level (mg./100 cc.)	Bromide Excreted (gm.)		
1	420	520	163	0.85	..	..
2	420	340	147	0.50	..	..
3	372	1,950	274	5.34	x	..
4	.....	1,050	450	4.72	x	..
5	.....	1,125	540	6.07	x	x
6	280	800	265	2.12	x	..
7	260	425	286	1.25	x	x
8	230	600	163	0.98	x	..
9	23 (?)	1,900	186	3.54	x	..
10	175	1,850	224	4.15	x	x
11	.....	.....	...	....	x	..
12	.....	1,920	100	1.92	x	..
13	114	3,800	109	4.14	x	x
14	83	775	83	0.64	x	..
15	75	1,400	75	1.05	x	..
16	67	2,950	67	1.97	x	x
17	.....	2,550	35	0.89	x	..
18	.....	3,850	28	1.07	x	..
19	45	3,350	53	1.77	x	x

the patient received no treatment. The bromide output was 1.18 gm. on the first day and 0.17 gm. on the second. On the morning of the third day, 2 cc. of mercuhydrin were injected. This increased the total urinary bromide output to 2.20 gm. On the third and fourth days the bromide output decreased to 0.48 and 0.82 gm., respectively, while on the sixth day, when mercuhydrin was again given, it totaled 2.35 gm. On the following day, 1.11 gm. were excreted. It is immediately apparent from the values in Table I that an increase in urinary bromide concentration occurred with each injection of mercuhydrin, accompanied by little or no increase in urinary volume. In summary, the average daily bromide output without mercuhydrin was 0.75 gm. and with mercuhydrin, 2.28 gm.—a threefold increase.

2. Does mercuhydrin increase bromide excretion when given in addition to standard sodium chloride therapy?

CASE II. On admission this patient (H. H.) was found to have a bromide level of 420 mg. per cent. (Table II.) On the first and second days he received no therapy and his daily urinary bromide excretion was found to be 0.85 gm. and 0.50 gm., respectively. Beginning on the third day he received sodium chloride, 1.5 gm. four times a day, and this was continued for the remainder of the observation period. In Table II the increase of bromide output on the third and fourth days can be noted. On the fifth day (which was the third day of sodium chloride administration) and every third day thereafter, he received 2 cc. of mercuhydrin by intramuscular injection. As indicated in Table II the daily bromide excretion increased with mercuhydrin, except for one apparently ineffective injection. The increased bromide excretion in this case apparently was the result of both an increased urinary concentration of the halide, together with an increase in the urinary volume.

3. Does mercuhydrin increase bromide excretion when given in addition to ammonium chloride therapy?

The investigation of this question was prompted by a recent report on the effectiveness of ammonium chloride administration in the elimination of bromides;[9] furthermore, by the frequent observation that ammonium chloride potentiates the effect of mercurial diuretics.

CASE III. This patient (M. J.) received ammonium chloride, 1.5 gm. four times a day, during the entire observation period. On the third and sixth days she was given 2 cc. of intramuscular mercuhydrin. In Table III note the marked increase of bromide excre-

TABLE III

BROMIDE INTOXICATION TREATED WITH AMMONIUM CHLORIDE AND MERCUHYDRIN

Case III (M. J.)

Day	Serum Bromide Level (mg./100 cc.)	Urine			NH_4Cl Administered	Mercuhydrin Administered
		24-Hour Volume (cc.)	Bromide Level (mg./100 cc.)	Bromide Excreted (gm.)		
1	.....	925	56	0.51	x	..
2	95	800	70	0.56	x	..
3	61	2,000	117	2.30	x	x
4	33 (?)	680	78	0.53	x	..
5	67	475	60	0.28	x	..
6	67	2,630	86	2.26	x	x
7	60	415	38	0.15	x	..

TABLE IV

BROMIDE INTOXICATION TREATED WITH ALTERNATING COURSES OF SODIUM CHLORIDE PLUS MERCUHYDRIN AND AMMONIUM CHLORIDE PLUS MERCUHYDRIN

Case IV (S. D.)

Day	Serum Bromide Level (mg./100 cc.)	Urine			NaCl Given	NH_4Cl Given	Mercuhydrin Given
		24-Hour Volume (cc.)	Bromide Level (mg./100 cc.)	Bromide Excreted (gm.)			
1	207	2,970	105	3.12	x	..	..
2	186	2,900	186	5.40	x	..	..
3	117	3,400	147	5.00	x	..	x
4	127	300	60	0.18	..	x	..
5	133	133	23	0.55	..	x	..
6	127	1,600	137	2.19	..	x	x
7	117	2,270	38	0.86	x	..	..
8	100	2,350	78	1.73	x	..	..
9	86	2,600	100	2.60	x	..	x
10	75	1,900	49	0.93	..	x	..
11	63	2,500	43	1.07	..	x	..
12	45	3,600	40	1.44	..	x	x

tion evoked by these injections. This resulted from an increased urinary volume together with an augmented urine bromide concentration.

After these preliminary observations were made the following routine regimen was established for the remainder of the patients. Three-day periods of sodium chloride administration were alternated with like periods of ammonium chloride. On the third day of each period, 2 cc. of mercuhydrin were injected. This schedule was adopted in order that an increased number of observations could be made on the effect of mercuhydrin using a relatively small group of patients. It also served to determine the relative value of the two regimens: a combination of mercuhydrin with sodium chloride and with ammonium chloride. Each patient served as his own control because of the two diuretic-free days in each three-day period and also by alternation of the treatment schedules. The results are shown in the following two illustrative cases:

TABLE V

BROMIDE INTOXICATION TREATED WITH ALTERNATING COURSES OF SODIUM CHLORIDE PLUS MERCUHYDRIN AND AMMONIUM CHLORIDE PLUS MERCUHYDRIN

Case v (J. M.)

Day	Serum Bromide Level (mg./100 cc.)	Urine			NaCl Administered	NH_4Cl Administered	Mercuhydrin Administered
		24-Hour Volume	Bromide Level (mg./100 cc.)	Bromide Excreted (gm.)			
1	284	1,130	205	2.32	x	..	..
2	244	1,100	336	3.70	x	..	x
3	210	975	163	1.59	..	x	..
4	180	1,100	236	2.60	..	x	..
5	157	2,020	217	4.38	..	x	x
6	175	710	109	0.77	x	..	..
7	86	615	186	1.14	x	..	..
8	147	1,440	180	2.59	x	..	x
9	100	560	198	1.11	..	x	..
10	127	750	123	0.92	..	x	..
11	92	1,700	168	2.86	..	x	x

CASE IV. The patient (S. D.) was admitted to the hospital with a blood bromide level of 207 mg. per cent (Table IV) and started on a three-day sodium chloride therapeutic regimen, alternating with three-day intervals of ammonium chloride. On the third day of each drug period mercuhydrin was administered intramuscularly. Table IV shows that the excretion of bromide was not increased by the first, but markedly increased by the second, moderately by the third, and slightly by the fourth mercuhydrin injection. Note again the increase of urinary bromide concentration with the second and third, and the urinary volume with the fourth injection of the drug. The blood bromide level fell from 207 mg. per cent to 45 mg. per cent in this eleven-day period.

CASE V. This patient (J. M.) was admitted to the hospital with a blood bromide level of 284 mg. per cent. (Table V.) Through a technical error his first sodium chloride treatment period lasted only two days. In Table V, note the increase of bromide excretion following every mercuhydrin injection, the increase of bromide concentration in the urine after the first, the increase of urinary volume after the second and third injections, and the increase of both volume and concentration after the fourth injection. The blood bromide level decreased from 284 mg. per cent to 92 mg. per cent in the nine-day treatment period.

RESULTS AND COMMENTS

The results of this investigation may be summarized as follows:

1. When mercuhydrin was combined with the administration of sodium chloride, renal bromide excretion was increased by an over-all average of 60 per cent, with a range from 14 per cent to 124 per cent in the individual patients. (Table VI.)

TABLE VI

SUMMARY OF THE EFFECT OF SODIUM CHLORIDE PLUS MERCUHYDRIN ON BROMIDE EXCRETION

Case No. and Patient	Average Bromide Excretion (gm./day)		Increase of Bromide Excretion by Mercuhydrin (%)
	NaCl Alone	NaCl plus Mercuhydrin	
I (C. W.)	2.50	2.85	14
II (H. H.)	2.23	3.22	44
IV (S. D.)	2.78	3.80	37
V (J. M.)	1.40	3.14	124
VI (E. C.)	1.51	2.86	89
VII (D. W.)	2.80	3.50	25
VIII (J. H.)	2.44	5.27	116
IX (J. S.)	0.88	1.54	75
Average	2.07	3.27	60

2. When mercuhydrin was combined with the administration of ammonium chloride, renal bromide excretion was increased by an over-all average of 130 per cent, with a range from 27

per cent to 470 per cent in individual instances. (Table VII.)

3. In the nine patients treated with mercuhydrin and sodium or ammonium chloride, the serum bromide level decreased from an average of 236 mg. per cent to 75 mg. per cent in nine days, an average of 18 mg. per cent fall per day. (Table VIII.) The symptoms of bromide intoxication disappeared by the time the blood bromide level reached 100 mg. per cent, which indicates that the average hospitalization required would have been less than nine days. Actually, most of the patients stayed longer than this for investigation of an underlying mental illness.

TABLE VII

SUMMARY OF THE EFFECT OF AMMONIUM CHLORIDE PLUS MERCUHYDRIN ON BROMIDE EXCRETION

Case No. and Patient	Average Bromide Excretion (gm./day)		Increase of Bromide Excretion by Mercuhydrin (%)
	NH_4Cl Alone	NH_4Cl plus Mercuhydrin	
III (M. J.)	0.40	2.28	470
IV (S. D.)	0.68	1.81	166
V (J. M.)	1.55	3.62	133
VI (E. C.)	1.89	2.39	27
VIII (J. H.)	1.70	3.08	81
IX (J. S.)	1.86	3.17	70
X (J. M., II)	1.11	2.32	109
XI (W. B.)	2.01	7.02	250
Average	1.40	3.21	130

TABLE VIII

SUMMARY OF THE EFFECT OF MERCUHYDRIN PLUS SODIUM CHLORIDE OR AMMONIUM CHLORIDE ON BLOOD BROMIDE LEVELS

Case No. and Patient	Blood Bromide (mg. %)		Length of Treatment in Days
	Initial	End	
II (H. H.)	372	95	11
III (M. J.)	133	60	3
IV (S. D.)	207	45	11
V (J. M.)	284	92	10
VI (E. C.)	244	95	9
VII (D. W.)	284	100	11
VIII (J. H.)	250	92	9
IX (J. S.)	133	21	8
X (J. M., II)	214	75	6
Average	236	75	9

4. As could have been expected, the combination of a mercurial diuretic with ammonium chloride has proved more effective than the combination with sodium chloride, due to the known potentiating effect of ammonium chloride on mercurial diuresis. An additional advantage of ammonium chloride over sodium chloride is that it can be administered to patients whose sodium intake must be restricted.

5. The following additional observations were made during this investigation: (*a*) The bromide output is independent of the total urinary volume. This was shown by the fact that increased bromide excretion was obtained when the bromide concentration in the urine was either low or high. This situation is analogous to the urinary excretion of chlorides under the influence of mercurial diuretics and suggests an identical mode of action of mercurials upon the two ions, namely, blocking of tubular reabsorption. (*b*) If given alone, sodium chloride was at least equally, and often more effective than ammonium chloride, in promoting urinary elimination of the bromide. This observation is in contrast to a recent observation that a more rapid decline in the blood bromide level occurs after ammonium chloride administration than with sodium chloride.[9] (*c*) Simultaneous bromide and chloride determinations in plasma and urine have shown that the preferential excretion of chloride over bromide by the kidney found in dog experiments by other investigators also holds for humans. We were unable to demonstrate, however, that the urinary bromide-chloride ratio was increased by mercurial diuretics.

6. This investigation suggests that combining ammonium chloride with a mercurial diuretic is the most effective method available for accelerating elimination of bromides from the body. The treatment schedule recommended is the administration of ammonium chloride, 6 gm. per day in divided doses, together with the intramuscular injection of 2 cc. of mercuhydrin every second or third day.

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Review

The Subjective Response and Reaction to Sensation*

The Reaction Phase as the Effective Site for Drug Action

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STUDY and measurement of subjective responses† have been carried on in our laboratory for some years. As a consequence of this work the conviction has gradually emerged that drugs which are effective in altering subjective responses act in large part by modifying the reaction to original sensation, rather than by direct effect on the original sensation. Reaction to sensation appears to be a very important, possibly the most important, factor in the stimulation-suffering sequence as far as the individual is concerned.

In an earlier paper[2] attention was drawn to the peculiar problems of definition and measurement of subjective responses as opposed to objective responses. That paper dealt primarily with matters of orientation, for example man as essential for study of subjective responses. It also dealt with the importance of the dual concept of initial sensation and reaction to the initial sensation. The view was also presented there that therapeutic agents designed to modify subjective responses arising in disease or trauma must be studied where they arise spontaneously, doubtless as a consequence of the very great importance in subjective responses of the reaction or "processing" component in their development and control. No convincing demonstration has yet been given that pathologic subjective responses can be usefully contrived; however, the essentiality of pathologic material is merely a useful working hypothesis, not yet disproved. The paper referred to also reviewed our method: the use of a group of cooperative individuals who report on the sensation under study. Arbitrary criteria of change in or relief of a disturbing symptom were set up. The necessary controls were described. These included the double "unknowns" technic, placebos, a standard of reference, correlated data, randomization of tests and mathematic validation of difference.

Although we have studied more than a score of subjective responses,[3] including pain, euphoria, dysphoria, drowsiness, relaxation, fatigue, sleep, nausea and so on, our principal work has been done on pain. We have not thought of the measurement of pain as only an end in itself. In addition to the theoretic and practical advantages accruing in such work, study of pain has been to us also a working model, a prototype, for study of ways and means of enquiry into other kinds of subjective responses.

An acceptable degree of accuracy can be achieved in the measurement of subjective responses. For example, we carried out the following experiment. Two series of flasks, six in each, contained unknown solutions. The task was to find which flask of one series was comparable in analgesic power to which flask of the other. At the end we[19] found one series had

† *Subjective responses* are symptoms. They are evident only to the individual experiencing them. They can be imparted to an onlooker through a cooperative statement by the subject. *Objective responses*, on the other hand, are made evident in physical change, or can thus be made evident, to the senses of an onlooker. They are physical signs. They can be mechanically recorded.

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contained always 10 mg. morphine per ml., and in the other series the concentration of morphine had varied. On graphing the paired doses against differential percentage of pain relief, we found the 10 mg. morphine of one series to be equivalent to 10.8 mg. of the other, an 8 per cent error. When the regression lines are calculated, this rises to a total error of about 10 per cent.

I shall state as exactly as possible what is meant by sensation and reaction. The output from the sensory receptors is the primary phenomenon and is derived from stimulation. The resulting afferent nerve impulse or impulses emerge finally in the central nervous system and become there a recognized sensation. Presumably in all normal individuals the primary, the initial events, is the same for a given stimulus. Also there can be little doubt that the secondary response which is the reaction to the primary or the processing of the primary event, is different for each individual. Cleavage between primary and secondary response has to be an arbitrary matter. From a neurophysiologic view it would seem better to place the end of the primary response just before any processing had begun. This has the objection that not only is one then obliged to consider unconscious sensory phenomena, but also in practical terms this is impossible. Practically it seems better to call the events including the eruption of the sensation into consciousness as primary, "the original sensation," and the succeeding events as secondary, as reaction, as processing. One must face the fact that processing probably begins before awareness has been achieved.

The existence of the sensation and its recognition then is the stimulus which precipitates the reaction and presumably the major part of the processing. In the sense in which we are using the term reaction we do not refer to physical activity such as the withdrawal of a burned finger from a flame, but rather to the mental process set up by the original sensation. It seems hardly questionable that this perception and process of recognition are influenced by the subject's concept of the sensation, by its significance, by its importance and degree of seriousness. An ache beneath the sternum in connoting death from sudden heart failure can be a wholly unsettling experience, whereas the same intensity and duration of ache in a finger is a trivial annoyance easily disregarded. It seems unquestionable too that the meaning of a sensation depends upon and is governed in large part by past experience as well as by present consideration; so discrimination, memory and judgment enter into the process of reaction. One can suppose that in physical terms, "association paths," "long circuiting," "reverberation" of nerve impulses and thus internuncial neurones are involved. We can reasonably extend this working hypothesis to suppose that when we are able to modify a subjective response by the use of drugs, that drugs are effective either by virtue of (1) lessening or blocking the original sensation or (2) by reducing or impeding the process of recognition or (3) by altering the processes of discrimination, memory and judgment which follow recognition.

Now to look back a little, the basic reason for the choice of the dichotomy, original sensation—reaction, has a rather long history; it goes back sixty years to a book by Marshall[23] for it was there that the concept of the reaction as important began to emerge. Marshall said " . . . I cannot bring myself to believe that . . . pains can be revived apart from any content to which they are attached." According to Marshall's theory, " . . . pleasure and pain are not independent mental contents, capable of existing in consciousness alone, but [are] . . . a sort of modification or coloring of sensations and ideas" (Strong, 1895). While Marshall did not clearly formulate the crucial assumption, Strong (1895) did, stimulated by Marshall. Strong said, "Whenever we feel a pain, there we have a sensation or idea, distinct from the pain, with reference to which pain is felt, . . . in every actual state of mind we are able to distinguish these two sides, the cognitive and affective."

It is an assumption, not more, that all pain experience in man consists of the original sensation plus the psychic reaction to that stimulus and we assume further that in various situations there are great quantitative differences in the role of each component; there is much to support this hypothesis. It is our view that this assumption can be extended probably to include all subjective responses, especially those that arise in disease or trauma. It is also our view (assumption) that because of the difficulty (or impossibility) of reproducing in the laboratory pathologic reaction to the original stimulus, the choice of "real" as opposed to contrived sensation is a good one. Hardy, Wolff and Goodell[15] say that "*pain sensation must be separated from associated reaction pattern* if progress is to be made." I agree that this separation is desirable, but

doubt that it has been made as yet, clearly and unequivocally, in work with experimental pain. Not much imagination is required to suppose that the sickbed of the patient in pain with its ominous threat against his happiness, his security, his very life, provides an entirely different milieu and reaction than the laboratory. Doubtless some anxiety and some fear can be contrived in the laboratory and associated with experimental pain. It is not likely that this contrived situation can ever be made to approximate the real situation which arises in disease states or trauma.

After this section was written it was of interest to find the following statement by Bishop.[9] "A comparison of the attitude of the subject undergoing pain stimulation as an experimental procedure with that of the sick and anxious patient whose pain is mysterious, unpredictable and of unknown causation, not to mention the factor of persistence of the pain, indicates that in causal experience the reaction to pain may be of more significance to the animal than its mere perception."

Of course the importance of the assumption hinges on the question of how great the reaction element actually is. There is reason to believe that it dominates, or at least can dominate the situation.

Hardy, Wolff and Goodell appear to believe they have in their experimental pain a pure culture of original sensation, so to speak, for they often write of their data as providing access to study of sensations without reaction and yet they go on to describe their own rather elaborate response (reaction) to their experiments. (See Wolff, Hardy and Goodell[27] pp. 664 and 677; Wolff and Wolf[28] pp. 10 and 14.) It is evident that they have had a decided reaction to the total situation. They seem to dismiss a pleasant reaction as no reaction at all, and in reaction appear to include only unpleasant responses.

While a good many different approaches to the study of subjective responses are possible, some appear more promising than others in terms of probable results. It has been a continuing source of surprise and wonder to me that the dichotomy mentioned earlier, experimental versus pathologic source of sensations, has not been an obvious distinction to make. It has not been to some. The matter is pertinent to the theme of this paper. Since a fundamental and, in my view at least, most important assumption is involved, one that throws some light perhaps on the phenomenon of perception, it may be well to summarize the reasons for this choice. If, in the end, this assumption is found to be as full of faults as an old shoe, it will be as easily discarded. As long as its results are as productive as at present,* it will be retained.

We[12] were unable to differentiate in man between 15 mg. morphine and a placebo with the Hardy-Wolff-Goodell method of producing pain with a measured amount of heat on the forehead, even after long and careful trial. An investigator with years of experience with that method who came to help us also failed, as long as he was kept in ignorance of which agent was used. So also other investigators failed to confirm the pain threshold changes reported by Hardy, Wolff and Goodell.[8,10,13,16,17,20,25,26] Failure has involved not only the Hardy-Wolff-Goodell technic but also other experimental pain technics in man.

So far as we have been able to determine, a large dose of morphine is not capable of significantly altering the brief jabs of experimental pain, even in properly set up and controlled experiments in man. Compare this with the fact that much smaller doses of morphine consistently reduce, often stop completely, the severe pain of an operative incision or a great wound. It seems to me that the sensible conclusion is that significantly the two situations are not comparable, and that something more than stimulation of nerve endings is involved, believed here to be reaction. *Great wounds with great significance and presumably great reaction are made painless by small doses of morphine, whereas fleeting experimental pains with no serious significance are not blocked by morphine. The difference here in the two situations would seem to be in difference of significance of the two wounds. Morphine acts on the significant pain, not on the other.*

Also related to this discussion is the question of why some wounds are painless and others are not. The total situation has of course great influence on the reaction that develops in it. Thus after removal from battle badly wounded men were often euphoric, their reaction to their wounds, to the removal from the battlefield (a milieu of destruction and death) to the relative safety of the forward hospital was one of happiness, nonetheless a pleasant reaction.[1] This

* We can successfully differentiate between powerful and weak analgesics and placebos as unknowns using pathologic pain, but not if we use experimental pain in man.

seems to be, and probably is, an example of a pleasant reaction having practical importance because a very high percentage in good general condition entirely denied pain from the extensive wounds or had so little pain they wanted no medication to relieve it. (Many had had no morphine.)

At the present time a contrasting study of wound pain is nearly completed. In this latter study a group of male civilian patients undergoing major surgery were asked the same questions as those put to the wounded soldiers. In the wounded soldiers about 25 per cent wanted medication to relieve such pain as they had and 75 per cent did not. In the civilians suffering from far less tissue trauma about 80 per cent wanted medication to relieve their pain and 20 per cent did not. Thus the figures are reversed. While the details are discussed elsewhere,[5] the important difference in the two groups seemed to lie in their responses to the wound. In the wounded soldier it was relief, thankfulness at his escape alive from the battlefield, even euphoria (his wound was a good thing); to the civilian his major surgery even though essential was a depressing, calamitous event. In the civilian group, pain was strikingly more frequent and more severe than in the soldier group. *These data state in numerical terms what is known to all thoughtful clinical observers: There is no simple, direct relationship between the wound per se and the pain experienced. The pain is in very large part determined by other factors, and of great importance here is the significance of the wound, i.e., reaction to the wound.*

Moreover there is the fact, widely reported and agreed upon, that experimental pain can be useful in appraising analgesic power in animals. In man, experimental pain has so far proved useless in the hands of many careful workers, but pathologic pain is highly useful for this purpose. Presumably pain is pain to an animal, and all pain serious and significant of danger. In man only pathologic pain is significant and serious. Thus in both instances *narcotics are effective but chiefly probably only in the presence of significant meaning of the pain involved. This looks as though narcotics are effective through their relationship to the meaning of the pain. This is just another way of saying the reaction to it.*

Consider also as recorded earlier[1] that the majority of men freshly and grievously wounded in warfare, but clear mentally, not in shock and with normal blood pressure, having had no narcotics for a period of four hours or more and some not at all, state on direct questioning that they do not have wound pain. *They complain as vigorously as normal men at an inept venipuncture; so there is no total pain block.* There is every reason to suppose that the wounds they have received stimulate sensory nerves, that the original stimulation starts out, but the usual end result has somehow been prevented. The usual response to a severe wound, pain, has not occurred in the majority of these cases. *Thus emotion can block pain; that is common experience. It is difficult to understand how emotion can affect the basic pain apparatus other than by affecting the reaction to the original sensation.* Certainly psychologic effects have great influence on subjective responses, not only pain but other responses as well. Every small boy has learned, knows, even though he does not consciously recognize the fact, that emotion can block the pain of a wound received during fighting but not perceived until the fight and the emotion have subsided.

Thus it seems reasonable to separate pain on the basis of its origin and significance to the subject, experimental or pathologic (this includes traumatic, of course). Presumably this applies to other subjective responses that have powerful connotations; this assumption needs further testing.

We do not pretend to know whether, in the foregoing instances, the pain sensation or the reaction to pain is blocked. However, since the conscious man badly wounded in warfare often does not suffer at all from his great wound, yet is annoyed by and apparently suffers normally from a venipuncture, one can conclude that the nervous system can transmit pain sensations but that somehow the reaction to them is the altered element.

Still another type of evidence supports the view that the most important factor in suffering is the reaction: Keats and Beecher[18] found that it was possible to differentiate between comfort and pain relief. Soon after initiation of that study, it was observed that in a sizable number of subjects following doses of morphine and more especially pentobarbital, the decision as to the presence or absence of pain relief was difficult. Two types of puzzling reactions were observed. One was in those subjects who claimed that their pain had not, or had only slightly, changed and

yet who did not want further medication. They appeared* comfortable, content and divorced from any "painful" experience in contrast to their pre-drug state. Despite the fact that their pain was said to be still present, we could not believe that further medication was indicated. The converse was found in those subjects who claimed that the pain was "quite a bit better," and yet who continued to be restless, tense, unhappy, bothered greatly by minor ailments (position, tubes) and generally uncomfortable. Here it was impossible to believe that the medication had been very successful, despite the relief of pain. The patient was not content. Therefore all doses were evaluated both for pain relief and for comfort. Thus four categories of response were observed: (1) no comfort, no pain relief; (2) no comfort, pain relief; (3) comfort, no pain relief; and (4) comfort, pain relief. The latter two categories of response were considered to represent the therapeutic or desired effect, both from the physician's and the patient's viewpoint.

For example, in 143 postoperative patients receiving intravenously 8 mg. morphine per 70 kg. body weight, twenty-seven obtained neither comfort nor pain relief, seven had pain relief but no comfort, nine had comfort but no pain relief and 100 obtained both comfort and pain relief from the medication. It appears to be possible and feasible to separate comfort and pain relief.

Somewhat comparable data were obtained following the intravenous injection of 60 or 90 mg. pentobarbital sodium per 70 kg. body weight. Here in 146 postoperative patients in pain, five had pain relief without comfort and sixteen comfort without pain relief. Presumably the comfort is established by the reaction. These "comfort" data offer suggestive support. This support appears somewhat stronger when it is recalled that the state produced by the use of these doses of barbiturate intravenously produce a state like that of the so-called prefrontal lobotomy. We even suggested that they produce a kind of pharmacologic lobotomy which as a guess may interfere with long circuiting of nerve impulses, association paths. It does not seem unreasonable to suppose that the reaction to pain requires the functioning of association paths, "long circuiting" of nerve impulses (cf. Keats and Beecher[18]). It is difficult to explain in any other way how frontal lobotomy or barbiturate can relieve pain (and this they have been shown to do) other than by altering the reaction to pain sensation. *Comfort and pain relief can be separated by a barbiturate, by morphine, and by prefrontal lobotomy. In the presence of apparently persisting pain ("my pain is the same, but it doesn't hurt me now") comfort can be established. The pain apparatus functions, but the disturbing element can be blocked in these three ways; evidently the processing, the reaction, is the altered factor.*

Further support for the importance of the reaction can be found in the effectiveness of antitussive drugs which so far in our studies[14] seem to be principally useful in altering the patient's state of mind and not his cough frequency (chronic cough). This work also supports the view that pathologic sources are essential for the appraisal of drugs designed to modify subjective responses arising there. While the effectiveness of antitussive agents is very slight in their effect on cough frequency (chronic cough), a suppressing trend seems to be present. This was not the case with experimental cough produced by the inhalation of ammonia gas or citric acid mist. It is interesting to observe in passing that experimental cough produced by the intravenous injection of paraldehyde (although not a satisfactory technic for reasons mentioned, *loc. cit.*) is associated with pain and fear (the other technics are not) and heroin was effective in reducing the number of paraldehyde induced coughs. This fits in with the picture already presented. *Study of cough shows that antitussive agents in patients with cough of pathologic origin do not significantly reduce the number of coughs, but the patients think they cough less. They can sometimes differentiate when tested with unknowns, between an "effective" antitussive, codeine and a placebo. In such cases the reaction, not the cough frequency, is modified by the antitussive agent.*

Stronger support for the importance of the reaction aspect of suffering than that in the immediately foregoing two paragraphs can be found in the repeated demonstration of the importance of placebos in relieving subjective responses. Over the years this placebo effect has been shown (by others in eight studies as well as ourselves in seven studies) to average 35 per cent of subjects relieved.[4] Since only some 75 per cent of patients in severe pain can be satisfactorily relieved[21] by even large doses of

* Here, of course, the judgment is based upon objective data.

morphine (15 mg. per 70 kg. body weight), this placebo effect amounts to about 50 per cent of the "drug" effectiveness. The only effect the placebo can have is on the reaction to pain. Certainly it would be impossible to believe that 1 ml. of normal saline had any physical effect on the anatomic apparatus of pain. *Placebos, organically ineffective as they are, can only affect reaction.*

In the work just referred to, the average effectiveness of placebos was mentioned as 35 per cent. In a study recently completed[6] it has been possible to show that the effectiveness of placebos is far greater when stress (pain) is greater than when it is less. When postoperative wound pain was at its greatest, a standard dose of morphine relieved 52 per cent of a group of subjects in pain; a placebo relieved 40 per cent of the same subjects, i.e., 77 per cent as many as those relieved by morphine. (Half of the population was given morphine first and half a placebo; at the second administration this order was reversed.) Later on, when the pain was much less in the same group of patients, the same dose of morphine relieved 89 per cent and the placebo 26 per cent. Cleghorn and his associates[11] in dealing with objective studies of the power of a placebo to fire the adrenals in anxiety states, reported that the effectiveness of a placebo increases, as measured by objective changes, with the degree of anxiety.

Once again in these observations we have a situation in which the most significance for the patient, whether pain or anxiety, is associated with the greatest placebo effect. *The increased effectiveness of placebos with increased stress can seemingly only be explained by the importance of the reaction or processing component of suffering.*

CONCLUSIONS

1. A considerable quantity of factual data has been presented here to support the sixty-year old speculations of Marshall and of Strong as to the existence (and importance) of the reaction or processing component in suffering.

2. In whichever way one looks at the problem of subjective responses, the reaction component looms larger in the stimulation-suffering-relief sequence than the original sensation. All of this leads to the practical conclusion that in treating subjective responses more attention might with profit be given to a search for therapy designed to alter reaction.

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Seminar on Allergy

The Genesis of Antibodies*

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An understanding of the genesis of antibodies would involve consideration of the nature of these substances, the processes by which they are synthesized, and the tissues or cells of the mammalian body involved in their production. Data which have been obtained in these areas in the case of the classic ("precipitating") antibodies will be presented here. It would not be consistent with the length or purpose of this review to attempt to refer to all of the literature which has been accumulated in this field. Of the investigations which have been reported, studies will be cited which initiated or are typical of the developments in these fields, or in which earlier literature on that investigative approach is presented.

CHARACTERIZATION OF ANTIBODIES

Since the recognition of antibodies as chemical entities in blood serum, data have been accumulated which point to their being similar in several properties to the serum globulins, especially to γ-globulin, but differing in the property of reacting specifically with their homologous antigens. Some of the evidence for this association of antibodies with normal serum globulin, and other relevant data, will be presented under three headings, physicochemical, chemical and immunologic.

Physicochemical Evidence for Identification of Antibodies with the γ-Globulins of Serum

Electrophoresis. The development by Tiselius of technics for electrophoretic examination of protein solutions was followed by the examination of sera in terms of the electrophoretic mobility of the plasma proteins, and then of antibody. In early studies[1,2] it was found that a number of antibodies of man, monkey and the rabbit migrate with the γ-globulin of the serum. Thus the total area under the γ-globulin peak was often found to be increased in animals being hyperimmunized and, more significantly, the area under this peak was found to be substantially decreased on specific removal of the antibody from the serum (absorption of the serum by the immunizing antigen). In the case of rabbits injected with egg albumin and then bled, the electrophoretically separated γ-globulin of the serum was found to constitute a fairly concentrated solution of anti-egg-albumin antibody.[1] Exceptions to the association of antibody with γ-globulin have, however, been noted. Thus antitoxic horse sera have been found to have an electrophoretic component not present in normal horse serum, with a mobility between that of β and γ-globulin, and the antitoxin has been found to be associated with this component.[1–4] Again, some antibody to tuberculin protein has been reported to be present in the α-globulin of rabbit serum.[5]

Ultracentrifugation. The analytic ultracentrifuge developed by Svedberg has made possible estimations of the molecular weight of proteins by the determination of the sedimentation constant. Study of antibody solutions prepared from antipneumococcal sera of rabbit, man and monkey, and of anti-diphtheria-toxin sera of the horse, have indicated that the molecular weights of the antibodies involved are similar to those of the γ-globulin of those species, in the order of 160,000, whereas other species, including the cow and pig, and the horse in the case of antipneumococcal antibody, were found to have antibody molecules of about five times that weight.[6] In the case of tetanus antitoxin in the horse this antibody was found to be largely in a molecular-weight fraction corresponding to γ-globulin, but with a fraction (10 to 20 per cent) of approximately double that molecular

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weight.[7] Human Wassermann antibody has also been found to be largely of the molecular weight of γ-globulin but with some of higher weight, in this case about six times as great.[8] The suggestion has been made that these higher molecular-weight species of antibody are polymers of units with the molecular weight of γ-globulin.[7,9]

Chemical Data on the Relationship of Humoral Antibodies to Serum γ-Globulins

The similarity between antibodies and normal serum γ-globulins has been studied by two types of chemical analysis; in the earlier literature, in terms of the relative concentrations of certain amino acid residues in antibodies and γ-globulins, more recently by application of the technic of end group analysis for the determination of sequences of amino acids within a peptide chain.

Data on the Composition of Antibodies. Chemical data on direct analysis of antibodies in comparison with γ-globulins in general is of somewhat limited value because this technic can disprove, but cannot prove, the identity of molecular species. Data have been obtained on the percentage composition of such relevant quantities as total nitrogen, amide nitrogen and the respective amino acids in serum γ-globulins and in antibodies concentrated by non-specific means or occurring in specific precipitates. No significant differences have been found by several workers between the percentage composition of antibodies and γ-globulins in terms of quantities such as those already mentioned.[10–12] Data on this point have been summarized by Marrack.[13]

End Group Analysis. The ingenious technic of end group analysis devised by Sanger makes it possible to determine the identity of the terminal amino acid at the free amino end of a peptide chain, such as a protein, and the sequence of a number of the amino acids at that end of the peptide chain. Using this technic Porter[14] examined rabbit serum γ-globulin and rabbit anti-egg-albumin and found in each case that a single chain existed in the molecule and that (as far as the exploration was conducted—five amino acids) the same amino terminal sequence was present in both serum globulin and the antibody. In view of the great number of random possibilities of sequences of amino acids in peptide chains this finding of the same terminal pentapeptide in both molecular species examined presents evidence for the similarity, in terms of the amino acid sequence, of γ-globulin and an antibody.

Immunologic Evidence of Association of Antibodies with γ-Globulin

Evidence of the association of antibodies with γ-globulin has been obtained by a number of workers in two types of experiments involving the use of immunologic procedures. In such studies normal serum globulin and antibody globulin have been used as antigens for injection into rabbits and the extent of their reaction and cross reaction with the resulting antisera has been studied. Wright[15] injected normal horse serum into the rabbit and was able, under given conditions, to precipitate the resulting antibody from the rabbit serum by the use of specific precipitates of various horse antisera and their respective antigens. In a study involving antibody globulin as the antigen Treffers and Heidelberger[16] injected into rabbits washed specific precipitates of horse-anti-pneumococcus type II antibody and its homologous antigen, and obtained sera in those rabbits from which the specific precipitates would remove antibody. By the quantitative agglutinin technic it was found that an equal amount of antibody could be removed from these rabbit sera by specific precipitates of horse antibody against two other pneumococcal types or against Hemophilus influenzae, or by solutions of the horse anti-pneumococcal antibodies.

The data referred to thus far must not be considered to imply identity of the structure of two given molecular species, serum γ-globulin and antibody. First, it must be pointed out that γ-globulin itself has been found by more refined physicochemical analysis to be inhomogeneous. Thus the serum protein which is characterized by migrating within a single electrophoretic boundary in the original Tiselius cell has been found by the boundary spreading technic of Alberty[17] to have components with a range of electrophoretic mobilities and isoelectric points. Again, fractions of bovine γ-globulin have been separated by the method of electrophoresis convection.[18] In fact, immunologic differences have been found between electrophoretically separated fractions of human γ-globulin, although each fraction could absorb antibodies against the whole γ-globulin.[19] In the ultracentrifuge human γ-globulin has been found to

give a pattern characterized by an asymmetric peak, indicating the presence of two components with a small but definite difference in mean sedimentation constant, and therefore in size and/or shape. This difference by ultracentrifugation was not found to be correlated with differences in electrophoretic mobility. Rather, all the fractions separated by electrophoresis convection were found to have asymmetric peaks by ultracentrifugation, suggesting the possibility of an even greater number of components.[20]

There is then evidence of a substantial degree of heterogeneity in what is called normal serum γ-globulin. There have also been a number of observations, such as those already referred to, of differences between the γ-globulins and various antibodies, or portions of given antibodies, in sera. Finally, it is possible within the course of immunization of a given animal to have variations in the ratio of the serum concentration of antibody with the physicochemical characteristics of γ-globulin to that of antibody of the same specificity but differing in electrophoretic mobility.[1–3]

Although these limitations and discrepancies must be borne in mind, the substantial majority of humoral antibodies which have been studied have resembled normal serum γ-globulins in physicochemical properties, and it is this similarity which has given rise to the theories concerning the mechanism of antibody formation which will be presented subsequently.

THEORIES OF THE MECHANISM OF ANTIBODY FORMATION

Within the past twenty-five years three theories have been proposed as to the mechanism of formation of antibodies. The first of these was suggested in fairly similar terms by Breinl and Haurowitz,[12] by Alexander[21] and by Mudd.[22] According to the theories of these authors, the antigenic material is brought to the site of synthesis of globulins, after injection into the tissues of the animal. In the subsequent synthesis of globulin the peptide chain is made up of those amino acids which would constitute the configuration most nearly complementary to the reactive sites of the antigen, and thus make for maximum interaction with the latter. In this manner specificity is conferred on the globulin so that it acquires the properties of antibody. Thus within a given species antibodies would differ from each other and from normal γ-globulin by the composition or sequence of amino acids. This aspect of these theories is not borne out by the experimental finding of Porter, already referred to, that the terminal pentapeptides of rabbit serum γ-globulin and of rabbit anti-egg-albumin were identical. This group of theories has, however, contributed to the literature a more concrete concept of complementary structure of antigen and antibody than was provided by the earlier hypotheses of Ehrlich and Bordet.

In a theory of antibody formation formulated by Burnet cognizance was taken of the relatively long periods after the injection of antigen that evidence of the formation of antibody can be obtained. This theory postulates that in the antibody-forming cell, antigen modifies a globulin-synthesizing enzyme so as to produce a proteinase which is not normally a constituent of the cell. This proteinase carries the specificity-determining configurations of the antigen and synthesizes globulin which reacts specifically with the antigen. The proteinase is also reproduced with reproduction of the antibody-forming cell so that antibody formation can continue after the antigen is no longer present in the tissues.[23]

The hypothesis of an inheritable proteinase, which adds to the complexity of this theory, was inspired by the long-continued production of antibody after apparently limited contact between tissues and antigen. It should be pointed out that the striking instances of persistence of antibody-formation are of antiviral antibodies, in situations in which the tissues have had, or can be presumed to have had, contact with living virus. Such agents may persist in some form for periods of considerable length in the host tissue. Accordingly, the instances of the longest apparent persistence of antibody formation may not, in fact, imply continued antibody formation in the absence of detectable antigen for as long as might seem to be the case. Continued formation of antibody after the last known contact between antigen and tissue is, however, a problem which must be considered, despite the possibility that antigen in amounts undetectable by present methods may suffice to stimulate antibody production.

The last theory of antibody formation to be mentioned is that of Pauling.[24] According to this theory polypeptide chains corresponding to the amino acid sequence of γ-globulin, the configuration of which has not yet become stabilized, are folded in the presence of antigenic

material in such manner as to bring about a configuration of the two ends of the γ-globulin molecule which is complementary to that of reactive groups of the antigen molecule. This theory is consistent with the data on the divalence of antibody, and with the observation mentioned regarding the similarity of the amino end of the peptide chain of γ-globulin with that of an antibody of that species. This theory assumes the presence of antigenic material during the period of formation of antibody.

These theories are concerned essentially with conference of specificity upon the molecule of antibody protein as it is formed. Another problem of interest is that of the synthesis of that protein molecule, whether from free amino acids or from large preformed peptide chains. In a recent study of this question Green and Anker[25] injected glycine labelled with C^{14} into rabbits, and approximately two days later injected ovalbumin into the animals. Subsequently C^{14} was measured in serum anti-ovalbumin (in specific precipitates) and in non-antibody globulin of these animals to determine the extent of incorporation of C^{14} glycine into these proteins. The C^{14} content of the anti-ovalbumin was found to be lower than that of the serum globulin, indicating that the glycine used in the synthesis of antibody had been obtained from the free glycine in the metabolic pool of the animal rather than from glycine already incorporated into normal serum globulin. The results of this study indicate that antibody is not made from preformed protein but is synthesized from individual amino acids.

It may be of interest to refer here to studies of protein synthesis in the case of adaptive enzymes, since in the case of these proteins, as in antibodies, the specificity of functions depends on configuration of the molecule. In two studies of synthesis of adaptive enzymes[26,27] evidence has been obtained that here too the enzymes have been synthesized from free amino acids rather than by conversion from pre-existing proteins.

In the theories of the mechanism of antibody formation which have thus far been suggested such important considerations have been emphasized as the complementariness of reactive sites of antigen and antibody, the persistence of antibody formation, and the adequacy of configurational variability to provide the range of specificity required. Precise mechanisms have not been suggested since greater understanding of the mechanism of the synthesis of proteins in general must precede the formulation of an adequate and useful theory of the formation of antibodies in particular.

CELLULAR SOURCES OF ANTIBODIES

Although most of the experimental data now available on this subject have been accumulated in the last two decades, there were earlier reports of data, often indirect in nature, relating some tissue or cell type to the production of antibodies. Thus cells of the reticulo-endothelial system, primarily macrophages, were suggested as a source of antibodies since it seemed plausible that a cell with phagocytic functions might elaborate antibody to the foreign materials ingested by it.

Lymphatic tissue, in particular the lymphocytes, was suggested as a site of antibody production by Hektoen[28] who irradiated rats with dosage sufficient to decrease greatly the volume of lymphatic tissues and found a marked decrease in antibody production in such animals. This observation was confirmed in rabbits by Murphy and Sturm,[29] with the additional refinement of using a dosage of irradiation which could cause substantial reduction in the amount of lymphoid tissue without producing changes in the bone marrow. This dose of irradiation sufficed to reduce the ability of the animals to produce antibodies. These authors found that the application of dry heat, which increased the volume of lymphatic tissue within the body, also increased the amount of antibody produced to injected antigens.

That plasma cells might be related to immune processes was suggested by Huebschmann[30] on the basis of histologic observations of the spleen during infections. Klein[31] and Arneth[32] maintained that plasma cells, especially those found in chronic diseases, represented functional states of lymphocytes which, because of local conditions, are related to processes of immunity. The association between hyperglobulinemia and an increase in plasma cells in patients with chronic infections, chronic liver diseases and myeloma led Bing[33] to postulate that plasma cells might form serum globulins.

A considerable number of studies have been carried out on local antibody formation in various parts of the body, such as the cornea and the skin.[34,35] In these studies the emphasis has been placed on the anatomic site rather than on the tissue or cells involved. Studies on the

local formation of antibodies, which will not be discussed here because of space limitations have been included in a comprehensive review by McMaster[36] on the sites of antibody formation.

The Macrophage

An early experimental approach to the role of the macrophage in antibody formation was suggested by the phagocytic properties of these cells. In these experiments massive injections of India ink or other particulate foreign substances were given with the purpose of blockading the phagocytic cells of the body, including the macrophages. Such animals on subsequent injection of antigen were found to produce substantially less antibody than did the normal controls.[37,38] Some investigators were not able to confirm this observation.[39]

Another experimental approach to the relationship of the macrophage to antibody production was that of Sabin[40] who injected a colored antigen (azo dye protein) into rabbits and noted that this colored material disappeared from the splenic macrophages of these animals just before measurable antibody to the protein antigen appeared in the serum. The time relationship of these phenomena was taken to imply that the macrophages had been the source of the antibody found in the serum.

Still another approach was made by extracting masses of these cells at the site of injection of antigen. Hartley[41] injected aluminum hydroxide gel intradermally into rabbits and produced cutaneous nodules which consisted of accumulations of macrophages. When a virus suspension adsorbed to aluminum hydroxide was injected into such a nodule, antibodies could often be detected in extracts of this tissue before their appearance in the serum. Hartley concluded that since macrophages were the predominating cell type in the nodule they were responsible for production of the antibody.

On the other hand Ehrich et al.[42] injected lanolin-oil suspensions of bacteria into the foot pads of rabbits and obtained an intense infiltration of macrophages at the site of injection. Homologous antibody was not found in extracts of this injection site, although it was found in extracts of the draining lymph node. In addition, no antibody was detected in peritoneal exudates rich in macrophages which were obtained after intraperitoneal injections of antigen. The observations which do not suggest antibody formation in the reticuloendothelial system are consistent with observations by Miller et al.[43] that liver cells and Kupffer cells failed to incorporate a C^{14}-labelled amino acid into γ-globulin. This finding would imply that the Kupffer cells were not able to synthesize γ-globulin under the conditions of the experiment.

Lymphatic Tissue and Antibody Formation

The studies by McMaster et al. provided clear experimental evidence of antibody production within lymph nodes and gave impetus to further developments in this area. In the first of these investigations cellular antigens were injected into the ears of mice and at various intervals thereafter the lymph nodes draining the sites of such injections were excised, pooled and extracted. These extracts were found to contain agglutinins to the cells injected, and in higher concentration in extracts of lymph nodes obtained only a few days after the injection than in the blood serum obtained at that time.[44] Analogous results were obtained in the case of vaccinia virus and neutralizing antibodies to it.[45] Observations of a similar nature were reported by Burnet and Lush[46] who infected mice with influenza virus by inhalation and then found neutralizing antibodies in the mediastinal lymph nodes. Ehrich and Harris[47] extended these observations by injecting cellular antigens into the hind feet of rabbits and at intervals thereafter collecting the popliteal lymph nodes, as well as lymph from the afferent and efferent lymph vessels of that node. Antibody to the injected antigen was found in extracts of the lymph nodes and in lymph collected from the efferent lymphatic of that node ("efferent lymph"). In the first day or two after the appearance of antibody the titer was often higher in these materials than in the blood serum at that time. Antibody was not found in extracts of the site of injection of antigen or in the afferent lymph, except in low titer after the serum concentration had risen to high levels.

Finally, Dougherty et al.[48] injected mice with large doses of sheep erythrocytes, pooled all available lymph nodes and spleens of these animals, minced the tissue and washed away intercellular material. In extracts of the pooled cell mass agglutinins and hemolysins to the antigenic material could be found in significant quantities.

In some studies on antibody production in lymphatic tissue homologous antibody could not be found in extracts of the lymph nodes or

lymph cells examined. Soloviev et al.[49] injected influenza virus into the foot pads of rabbits and found no antibody in extracts of the inguinal lymph nodes. Habel et al.[50] failed to find substantial differences in extractable antibody between popliteal lymph nodes homolateral and contralateral to the foot pad injected. Craddock et al.[51] injected typhoid bacilli parenterally on two successive days and two days after the second injection collected lymph from the thoracic duct; homologous agglutinins could not be found in extracts of this lymph Finally, Erslev[52] injected rabbits intravenously with suspensions of pneumococci and collected blood from the animals at intervals during the course of injection. Antibody was not found in extracts of white cell suspensions of these blood specimens.

In a number of the studies cited factors involving the anatomic or temporal relationship between the injection of antigen and the lymphatic tissue examined for antibody might have accounted for the failure to obtain evidence of antibody formation. Consideration of such relationships is essential for this demonstration because of the wide distribution of lymph nodes throughout the body, as has been pointed out elsewhere.[53] In a number of recent studies performed for cytologic or other reasons homologous antibodies in lymph nodes draining the sites of injection of antigens have been found consistently.[54–59]

The volume of data relating the spleen to antibody formation is too great to be reviewed here. The work of Pfeiffer and Marx[60] in 1898, and others of the same period, and of Topley[61] considerably later, suggested a function of the spleen in the formation of antibodies. This function of the spleen, particularly following intravenous injection of antigen, has been established by studies involving extraction of this organ, splenectomy and irradiation before or shortly after the injection of antigen. An account of these studies has been included in the review by McMaster.[36]

Experimental work on antibody production by thymus tissue will be mentioned only briefly. Although the thymus is generally considered a part of the lymphatic system, this relationship is not certain. There is no general agreement among histologists as to whether the thymocytes are identical with lymphocytes, and the thymus does not show follicular organization characteristic of the cortex of lymph nodes and white pulp of the spleen. In studies by extraction of thymus tissue following parenteral injection of antigenic material homologous antibody was not found in such extracts by Harris et al.[62] or by Bjørneboe et al.[70] In studies by Fagraeus[71] and by Thorbecke and Keuning[77] of *in vitro* incubation of tissues of injected animals, explants of thymus were not found to produce antibodies under these circumstances. Finally, in studies of antibody formation by the transfer of cells and tissues Harris et al.[116,117] did not find evidence of antibody formation by cells obtained from the thymus. However, Hale and Stoner[109] have recently reported data suggesting antibody production by transplanted fragments of thymus.

The observations that lymphatic tissue could be a site of formation of antibody have led to a substantial amount of investigation as to the cell type or types within lymph nodes and spleen which might be involved in synthesis of the antibody formed there. Data have been obtained by a variety of experimental approaches but these have been in almost all cases in relation to the lymphocyte or the plasma cell, or to predecessors of these cell types. The discussion which follows is organized according to the type of experimental approach.

Studies on Relationships of Lymphocyte or Plasma Cell to Formation of Antibodies

Correlation of Tissue-Extract Antibody with Cytologic Observations. A number of earlier experimental observations indirectly suggested a relationship of the lymphocyte to the process of antibody formation before the first direct comparison of extractable antibody and cytologic composition of any tissue was made. Hellman and White[63] examined the spleens and lymph nodes of rabbits in relation to the injection of antigens and found that following such injections there was a marked increase in the number of germinal centers (nodules of early members of the lymphocytic series, surrounded by lymphocytes). In the lymphatic tissue of guinea pigs reared in a sterile environment Glimstedt[64] found no germinal centers; these were, however, numerous in such animals after exposure of the animals to bacteria. Oesterlind[65] found many such germinal centers in lymph nodes draining sites of injections of diphtheria toxoid in the rabbit, and the greatest number of these centers was observed at the time of highest serum antitoxin concentration. Finally, Rich et al.[66] were able to show that the "acute splenic tumor" which had been described in infectious diseases could be

induced in experimental animals by the injection of non-bacterial foreign protein, and that the predominant, proliferating cell type was the lymphoblast. They also found that lymph nodes draining a site of injection of foreign protein exhibited a "lymphoid cell proliferation entirely like that of the acute splenic tumor," and they called attention to the possible relation of these lymphoid cells to immunity.

Among studies involving the extraction of tissues and titration of antibody in such extracts for correlation with cytologic changes is that of Ehrich and Harris[47] already referred to. Antityphoid agglutinins were found in extracts of the popliteal lymph node on the third day after the injection of antigen, the highest concentration being reached on about the fifth day. Histologically, two days after the injection of antigen the cortex of the lymph node had become "tremendously enlarged and consisted of a diffuse lymphoid tissue which contained many large lymphocytes and mitotic figures." A few days later the diffuse hyperplasia of the cortex had become organized into secondary nodules. In the efferent lymph the cell count rose from a pre-injection level of 16,000–18,000/cu. mm. to a range of 40,000–150,000 on the fourth to sixth day. Of these cells the average percentage of small lymphocytes was 93 and that of large lymphocytes 6, or a total of 99 per cent. At this time the agglutinin titer of the efferent lymph was at its peak. In a study by Harris and Harris[67] of the formation of antihemagglutinin to influenza viruses in the popliteal lymph node similar histologic changes were observed, as well as similar time relations of the cortical hyperplasia to the maximum value of extractable antibody from that node. Finally, in the work of Dougherty et al.,[48] already referred to, it was reported that of the minced lymph node cell mass in which antibody had been found over 90 per cent of the cells were lymphocytes.

In studies of the plasma cell, also, there were experimental studies which preceded those involving extraction of tissues or cell masses. In 1938 Kolouch[68] examined bone marrow biopsy specimens of rabbits rendered "allergic" to Streptococcus viridans. The condition of the bone marrow was observed after the intravenous injection of a "shock dose" of the antigenic material. Within a few hours after this injection the bone marrow showed a great increase in plasmacytic reticulum cells, and also transformation of these cells into plasma cells; and after five days small plasma cells were predominant in the bone marrow. It was suggested that there might be an association between the cell transformation observed and the development of antibody.

Bjørneboe and Gormsen[69] brought about hyperglobulinemia in rabbits by intensive immunization with different types of pneumococci and found a concomitant increase in plasma cells in different tissues, which was apparently proportional to the concentration of antibody protein. They suggested that the increase in the globulin fraction of the blood was due to the increase in antibodies and that this, in turn, was related to plasmacytosis. In a later study involving the extraction of various tissues Bjørneboe et al.[70] gave rabbits many injections of large doses of pneumococci and produced cellular infiltration of the fat of the renal sinus. This tissue, which was estimated to contain 90 per cent plasma cells and 10 per cent lymphocytes, was extracted and the extracts were found to contain considerable amounts of antibody to the injected antigen. On the basis of these and earlier findings it was considered that antibodies are produced by plasma cells.

Cytologic studies of the development of plasma cells in lymphatic tissue following the injection of antigen may be discussed here. In these investigations antibody determinations were carried out only in the blood serum and not in extracts of the tissues examined. Fagraeus[71] gave rabbits repeated injections of bacterial or protein antigens and studied the cytology of the spleen in relation to the development of antibodies in the serum. It was found that reticulum cells appeared in the spleen slightly before the appearance of detectable serum antibodies (which was on the second or third day). These cells showed differentiation to immature plasma cells at the time of the most rapid rise in serum antibody concentration. This was followed by a gradual increase in the number of mature plasma cells. The author concluded that the formation of antibodies occurs with development of the reticulum cells into plasma cells. Ringertz and Adamson[72] studied, by means of differential cell counts, the cellular changes in lymph nodes both regional and non-regional to sites of injections of various antigens. The cellular response was studied in terms of a number of cell systems, the reticulum cell, the granulocytic, the lymphocytic and plasmacytic. The authors de-

scribed a strong lymphocytic as well as plasmacytic reaction in response to the injections of antigen. In discussing the possible role of the nodular centers (of lymphocytes) which were found the authors concluded as follows: "In the light of recent investigations on the role played by the plasma cell as an organ of antibody-formation it seems improbable that the centres have such a function. However, if it is true that the immature lymphocyte is the matrix of the plasma cell, it might be suggested that the centres play an indirect part in the antibody-formation, that is to say, they provide a large number of immature lymphocytes which under the influence of antigen are being converted into antibody producing plasma cells instead of, as is normal, developing into mature lymphocytes."

Marshall and White[73] found that intravenous injection of antigenic material in the rabbit caused a stimulation of primitive reticular cells which led to the formation of plasma cells in the spleen and also in the lungs, liver and bone marrow. The formation of germinal follicles was also noted but this was confined to the spleen.

Extraction of Cells. In the studies in which the foot pads of rabbits were injected and lymph collected from the efferent lymphatic vessel of that node it was possible, because of the volume of "efferent lymph" obtained and the relatively high cell count, to collect these cells, determine their volume by hematocrit, extract them in a given volume of diluent, test the resultant extracts for their content of homologous antibodies, and thus estimate the concentration of the antibodies within the cells. In experiments involving typhoid bacilli, sheep erythrocytes and influenza virus it was found that on the fifth day after the injection of antigen the antibody content of the cells of the efferent lymph was of a concentration ranging from four to twelve times that found in the lymph plasma from which the cells had been obtained. Data of other experiments indicated that these cells did not absorb this antibody from the surrounding lymph plasma. It was considered rather that these cells were a primary site of the antibodies found in the lymph.[67,74] According to differential cell counts carried out in one of these studies, and in a previous study of the series,[47] lymphocytes comprised on the average 99 per cent of the cells in such lymph specimens.

Release of Antibody from Tissues and from Cells Maintained in Vitro. Formation of antibody in tissue culture has thus far been reported as possible only when the explanted tissues were obtained from animals previously injected with antigen. This approach has been used in a number of recent studies on the cellular source of antibodies. Fagraeus injected rabbits repeatedly with living typhoid bacilli, then excised the spleens and separated as far as was possible the red pulp (in which the plasma cells are relatively more concentrated) from the white pulp (which contains the follicular structure and is therefore relatively richer in lymphocytes). Bits of each of the two types of splenic tissue were maintained at 37°c. in tissue culture medium. The agglutinin titers of extracts of the cultivated tissue were then determined. It was found that these titers were significantly higher than the antibody titer of control explants maintained in medium to which toluol had been added, or of extracts of similar bits of tissue kept at 4°c. Of the cultivated tissues only the fragments of red pulp were found to produce antibody. This was attributed by Fagraeus[75] to the presence of transitional cells and immature plasma cells in the red pulp. In an experiment of this series in which cytology was reported, imprint smears of the explants of red pulp showed a range of 0.4 to 4.4 per cent for transitional cells, 0.8 to 5.8 per cent for immature plasma cells, and 0.3 to 2.9 per cent for mature plasma cells. The greater production of antibody in some of these cultures was correlated with the development of immature plasma cells from transitional cells. No details were reported as to the percentage distribution of cells other than those cited.

Keuning and van der Slikke[76] studied the *in vitro* production of antibody by splenic tissue of rabbits which had been repeatedly injected with antigen, in a system generally similar to that of Fagraeus. They were able to confirm Fagraeus' finding of antibody production in cultures of red pulp but they also found evidence in some experiments of production of antibody by the explants of white pulp as well. In the case of the red pulp the production of antibody was attributed to plasma cells; in the case of explants of the white pulp cytologic study led these authors to associate the antibody produced with "immature lymphoid cells," either lymphoblasts or reticular lymphocytes. These authors suggest the possibility that following antigenic stimulation lymphoblastic cells give rise to both lymphocytes and plasma cells.

In a later study Thorbecke and Keuning[77] compared the production of agglutinins in roller

tube cultures of fragments of liver, thymus, bone marrow, lymph nodes and spleens removed from rabbits three days after the last of a series of repeated subcutaneous and intravenous injections of paratyphoid B vaccine. The authors found little or no antibody production in cultures of the liver or thymus, and no plasma cell reaction in either case. In bone marrow, lymph nodes and spleen (red and white pulp) antibody production was shown and appeared to be correlated with a higher number of plasma cells. The finding in this study of antibody production in the white pulp of the spleen was explained by aggregates of plasma cells contaminating the white pulp.

A different approach employing *in vitro* technic was made by Wesslén[78] who injected rabbits repeatedly with typhoid bacilli and then collected thoracic duct lymph from those rabbits. Washed cells from this source were maintained *in vitro* under conditions of tissue culture. The culture fluid was found to contain antityphoid agglutinins in a range of eight to eighty times that found in control preparations made with lysed cells. The cells in cultured preparations contained 5 to 6 per cent large lymphocytes, all the other cells being small lymphocytes.

Recently Stavitsky[79] reported on the maintenance *in vitro* of fragments of lymph nodes from rabbits injected in the foot pads at least twice with diphtheria toxoid. The antitoxin content of cultures after incubation was eight to ten times that of control cultures (in distilled water, or in the presence of inhibitors of oxidative metabolism).

Studies Involving Aggregation of Bacterial Cells Around Tissue Cells. Reiss, Mertens and Ehrich[80] injected typhoid organisms or brucella into rabbits feet, excised the lymph nodes and prepared cell suspensions from them. When such washed cell suspensions were added to suspensions of the organisms used as antigen, aggregation of the organisms about some of the cells was observed. Many of the agglutinating cells were plasma cells but only some of the identifiable plasma cells showed this. The cell which seemed to exhibit this phenomenon most often was a small cell too poorly differentiated for identification. The authors concluded that "the nature of this cell could not be established. In view of all other findings, however, it may be assumed that it belonged to the plasma cell series."

Hayes, Dougherty and Gebhardt[81] injected bacterial suspensions into skins of mice, then excised the site of injection and added suspensions of the bacteria to air-dried loose connective tissue and to imprints obtained from the tissue excised. They observed bacterial aggregates adhering to the cells of fragments of subcutaneous tissue. These cells were identified as lymphocytes.

Moeschlin and Demiral[82] reported the agglutination of organisms by cells of the spleens of animals repeatedly injected with bacterial antigens. By phase microscopy it was found that the cells which agglutinated the organisms contained cytoplasmic granules which the authors had previously described in developing plasma cells.

In each of these three studies the only control system used was that of cells from uninjected animals and the bacterial suspensions. No control experiments were reported involving cells from the injected animals and heterologous antigenic material, to test for the specificity of the reaction. Such controls would be of special importance in this experimental situation for the following reason. The specific agglutination of bacteria by washed tissue cells would require an assumption either that sufficient antibody is present in the wall of the tissue cells, which would not seem likely, or that long-range specific forces act between the bacteria and antibody present within the cells. It should be noted in this connection that substantial evidence against the existence of long-range specific forces has been presented by Karush and Siegel[83] and by Singer.[84]

Histochemical Staining for Nucleic Acid in Lymph Nodes in Relation to Formation of Antibodies. Caspersson,[85] Brachet[86] and others have obtained data suggesting an association of protein synthesis in cells with ribonucleic acid–containing nucleoli and cytoplasmic granules. Since protein is being synthesized in a lymph node which is producing antibody, studies have been conducted in which identification of the cell type producing the antibody within that lymph node was sought by histochemical tests for nucleic acids in popliteal lymph nodes draining the site of injections of antigens. Two such studies were reported simultaneously. In both of these analyses of extracts of the whole node for the two nucleic acids indicated an increase of ribonucleic acid (RNA) relative to the wet weight of the node, without a corresponding

increase in the desoxyribonucleic acid (DNA) content of the node. This increase was noted on the third day after the distal injection of antigen and was maximal at about the fifth day.

As to the cell type which was involved in this increase of RNA, as judged by histochemical stains for that substance (e.g. pyronine) in sections of the lymph nodes, the two studies were not in accord. In the study of Ehrich et al.[87] the medullary cords and the cortical tissue adjacent to them were found to contain many immature lymphoid cells with heavily pyronine-stained cytoplasm two days after injection of the antigen. On the fourth and especially on the fifth day this part of the node (medullary cords and adjacent cortex) showed predominantly mature plasma cells. Thereafter the number of plasma cells decreased. The cortex was found to be considerably enlarged on the fourth day, with many immature lymphoid cells which had pyronine-stained nucleoli and cytoplasmic particles. However, the cytoplasm of the lymphoid cells was found to have less intensely pyronine-stained structures. The authors compared the time of highest concentration of RNA and of mature plasma cells observed in this study with the time of maximum antibody production in the lymph node, which had been determined in an earlier study, and concluded that the antibody was formed by plasma cells.

In the other histochemical study, that of Harris and Harris,[88] cytologic observations were concentrated on the cortex, that part of the node which, as had been noted in an earlier description,[47] was "tremendously enlarged" in the reaction of the lymph node to injected antigen. Two days after the injection of antigen the cortex showed an intense and diffuse hyperplasia of young lymphocytes, interspersed with many reticulum cells containing pyronine-stained nucleoli and cytoplasmic particles. On successive days the cells containing these pyronine-stained structures became much more numerous and included many transitional forms with denser nuclei. By the fifth day the pyronine-stained cells were indistinguishable from the lymphocytes about them, except for slightly larger nuclei. At this time organization of the diffuse lymphoid hyperplasia into follicular structure was beginning to occur. The finding of pyronine-stained granules in this sequence of cells strongly indicated a gradual transition from reticulum cell to lymphocyte. (The maturation of lymphocytes from reticulum cells has also been observed in cultures of lymph node slices on the chorioallantoic membrane of hens' eggs.[89]) It was concluded in this study that the lymphocytes of the cortex, or transitional cells in the lymphocytic series, were probably the source of the antibodies found in these lymph nodes.

Histochemical Staining for Antibody. In recent years there have been several applications of a method elaborated by Coons of detecting antigens in tissues. In this method sections of tissues from animals injected with antigens are immersed into solutions of the homologous antibody which has been conjugated with fluorescein. On microscopic examination fluorescence is detectable at points in the tissue where the concentration of antigen is sufficient to cause combination with the fluoresceinized antibody. This technic has recently been applied by Coons to the detection of antibody in tissue. For the detection of antibody it was found necessary to alter the procedure as follows: The section of tissue containing antibody is first immersed in a solution of the homologous antigen, allowing an antigen-antibody precipitate to be formed at those points where the antibody is sufficiently concentrated in the tissue. The slide is then immersed in a solution of fluorescein-conjugated antibody, where the antigen which has been precipitated on the tissue antibody can combine also with the fluorescent antibody, thus marking the original site of antibody in the tissue. The method is less sensitive as applied to the detection of antibody than of antigen because of the greater concentration of antibody than antigen in specific precipitates.

This technic of detecting antibody in tissue was applied by Coons et al. to a study of the cellular source of antibodies. Since cells cannot be identified histologically in "fluorescence-stained" sections, attempts were made in this study to stain sections first with the fluorescent reagent and then with histologic stains. This proved not to be feasible, so given sections of the tissue were treated with the fluoresceinized antibody and adjacent ones were stained for histologic study. With this technic the following observations were made: In animals which had received two or more injections of antigen, groups of fluorescence-stained cells were found in the red pulp of the spleen (following intravenous injection)[90] or in medullary cords of popliteal lymph nodes (after foot-pad injection).[91] These groups of cells were identified as plasma cells by cytologic study of the sections of adja-

cent tissue. Fluorescence of substantially lower intensity was occasionally observed in cells of the lymphoid follicles. In the spleens of the intravenously injected animals these were noted as faint traces of fluorescence in the cytoplasm of the larger, more angular cells, with larger nuclei than the cells of the red pulp. In the animals which had received the injections of antigens in the foot pad an occasional rabbit showed a small number of antibody-containing cells within the area of some of the follicles of the draining lymph nodes. In the case of lymph nodes draining the site of a single injection of antigen, cells showing fluorescence appeared on the fourth day and were relatively rare, typically ten to twenty such cells in a whole section. These were "large cells, with a thin rim of faintly to moderately brightly fluorescent cytoplasm, scattered singly in the medullary areas near the edges of the follicles or in the medullary cords." On the sixth to eighth day the number of cells showing fluorescence "had increased to about 50 or more mature cells, some of them mature plasma cells (under the fluorescence microscope)."

In these studies, as the authors state, "the identification of the cell type responsible for the bulk of antibody production depends on the circumstance that during the secondary response, or in animals a few days after the last of a series of repeated injections, antibody-containing cells are present in large groups." These are the groups of plasma cells previously referred to. The authors conclude that the plasma cell was the source of the antibody found in these preparations but that a minor contribution of lymphocytes to antibody synthesis could not be excluded.

Transfer of Cells of Lymph Nodes, Lymph and Spleen. A technic which has been applied in recent years to the study of some aspects of antibody formation is that of the transfer of cells from tissues presumably engaged in the formation of antibodies (usually from donor animals injected previously with antigen) to recipient animals (which have not had contact with the antigen). Landsteiner and Chase[92] described the passive transfer to normal guinea pigs of hypersensitivity to simple chemical compounds by the transfer of cells obtained from the peritoneal exudates of highly sensitive donor guinea pigs. Chase[93] succeeded in transferring hypersensitivity to tuberculin by means of cells from peritoneal exudates, lymph nodes and spleens of tuberculous guinea pigs. This has been confirmed by many workers.[94–100] In 1950 Chase[101,102] transferred hypersensitivity to picryl chloride to normal guinea pigs by injection of cells of peritoneal exudates, lymph nodes and spleens of guinea pigs highly sensitive to picryl chloride. The recipient animals developed skin sensitivity and anaphylactogenic antibodies to picryl chloride. If in such experiments donor animals were injected with sheep erythrocytes, hemolysins could be found in the sera of the recipient animals. Extracts of the cell suspension and cell suspensions which had been frozen and thawed failed to produce the effect. Subsequently the technic of cell transfer has been used in the study of the formation of humoral antibodies to a variety of antigens under different experimental conditions. The technic has also been applied to studies in hypersensitivities of different types but these do not fall within the scope of this discussion.

Transfer of cells from donors injected with antigen: Harris et al.[103,104] injected dysentery bacilli into the feet of rabbits, excised the draining popliteal lymph nodes, and transferred suspensions of cells obtained from these nodes into fresh rabbits. Agglutinins to dysentery bacilli appeared in the sera of the recipients in a characteristic pattern. Agglutinins did not appear in the sera of recipients of lymph node cells which had been injured by repeated freezing and thawing, heating, etc. prior to transfer. Wager and Chase[105] and Stavitsky[106] reported the appearance of diphtheria antitoxin in recipients of cells obtained from spleens and lymph nodes of immunized donors. Wesslén[78] obtained lymphocytes from the thoracic duct of rabbits sensitized to horse serum; when such cells were transferred to guinea pigs they reacted with severe anaphylactic shock when injected with 0.3 ml. of horse serum one to four days after transfer.

In studies of immunity to transplantable tumors Mitchison[112,113] obtained cells from mouse lymph nodes regional to tumor homografts and transferred them, either minced or in suspension, to a secondary host. It was found that the transferred cells had the capacity to confer immunity on the recipient if the nodes were removed from the donor mouse at the time the grafted tumor was undergoing breakdown. Cells from other lymph nodes and from the spleen, as well as serum or whole blood, failed to transfer immunity. Evidence was presented favoring the hypothesis that "the lymph node

cells were immunologically activated before transfer, and that they conferred immunity by continuing to function in their host." Homografts of a transplantable sarcoma gave rise to the production of serum antibody which could be detected by its cytotoxic action on the cells of the tumor and also by means of a hemagglutinin test. Following the transfer of lymph node cells from mice with such grafts into hosts of the same strain hemagglutinin could be detected in the host serum. The capacity of the cells to transfer hemagglutinin production developed later than the power to transfer increased resistance to grafts. Splenic cells also transferred hemagglutinin production, although to a lesser extent. The conclusion was drawn that the hemagglutinating antibody is distinct from the antibody effective in protection against homografts.[114]

Transfer of tissue fragments of lymphatic and other tissue: The transfer of tissue fragments from donors injected with antigen to fresh recipients was reported as early as 1930. Topley[61] minced spleens of rabbits injected intravenously twenty-four hours earlier with paratyphoid bacilli and injected the tissue fragments intraperitoneally into fresh rabbits. The sera of the recipients developed agglutinins within a few days after transfer, which was earlier than would have been expected in the case of a primary response of the recipient to antigen present in the transplanted tissue. Fagraeus and Grabar[107] transplanted fragments of splenic tissue from immunized donors into the peritoneum of recipients and subsequently found antibody in the serum of the latter. Antibody was not detected after the transfer of splenic tissue which had been suspended in distilled water.

Hale and Stoner[108] obtained fragments of lymph nodes and spleen of mice which had received two injections of tetanus toxoid and transplanted these into the anterior chamber of the eyes of irradiated mice. Antitoxin appeared in the sera of the recipients in low concentration. If the recipients were injected intravenously with tetanus toxoid ten days after the transplantation their sera developed higher levels of antitoxin. If the transplanted tissue was frozen and thawed prior to transplantation no measurable antitoxin developed in the sera of recipients. In a later study employing the same experimental approach[109] these authors reported that following transplantation of fragments of thymus and Peyer's patch obtained from mice injected with alum-precipitated tetanus toxoid subcutaneously and intra-abdominally, antitoxin could be detected in the sera of irradiated recipients if the recipients were injected intravenously with fluid tetanus toxoid after transplantation.

In a study on antibody production in transplants by Oakley et al.,[110] rabbits were injected with diphtheria and/or tetanus toxoid and one month later the same antigens were injected into the interscapular fat or into the hind feet of these animals. At various intervals thereafter the interscapular fat or the popliteal lymph nodes were transplanted into the omentum of normal rabbits. Antitoxins to the respective antigens appeared in the sera of the recipients. Pieces of fat which were ground before transplantation (as controls) did not give rise to the appearance of antitoxin in the same manner. In a series of experiments in which transplants were made within a range of three to ten days after the secondary injection of antigen it was found that antitoxin appeared in the serum of the recipient at a given interval after the injection of antigen into the donor, regardless of the time of transplantation. This finding is similar to one which has been described in studies by cell transfer of antibody production to the agglutinogen of Shigella paradysenteriae.[111] Oakley et al. found, however, that antitoxin could be detected subsequently in the recipient only if the rabbit fat had remained in the donor for at least twenty-four hours after secondary stimulation before it was transplanted. Because of this the authors conclude that the injected mass of fat, while *in situ* in the donor, is infiltrated with antitoxin-producing cells. In experiments with lymph node cells, on the other hand, a period of ten minutes between the injection of antigen into the donor and the collection of its cells has been found adequate. When such cells, from lymph nodes regional to the site of injection, were transferred to irradiated recipient animals agglutinin appeared subsequently in the sera of the latter.[110]

Oakley et al. found that heterologous transplants (guinea pig fat tissue into rabbits and horse popliteal glands into rabbits) do not continue to produce antitoxin in the recipient. This finding is similar to those of Chase[101] and of Harris et al.[103] Wesslén,[78] on the other hand, has described successful transfer of washed lymphocytes of thoracic duct lymph of rabbits to guinea pigs.

Transfer of cells to x-irradiated recipients: In the course of determining the optimum interval between the injection of dysentery antigen into the donor and the collection of its lymph node cells, Harris et al.[111] found it necessary to eliminate the possibility of active formation of antibody by the tissues of the recipient.[115] For this purpose they employed irradiation of the recipients twenty-four hours prior to transfer. It was found that when lymph node cells of uninjected donors were transferred to irradiated recipients, and immediately thereafter dysentery antigen was injected into the same animal, agglutinins to dysentery bacilli appeared in the sera of such recipients a few days later. Irradiated rabbits which had received heated cells and antigen, or antigen alone, did not develop agglutinins in this period.[116,117]

Roberts and Dixon[118] transferred to irradiated recipients lymph node cells (popliteal, mesenteric and axillary nodes) from donor rabbits immunized over a five-week period with bovine γ-globulin or bovine serum albumin. The recipients were then injected with radioiodinated bovine γ-globulin or with bovine serum albumin (I*BGG and I*BSA) respectively. The rate elimination of I*BGG and I*BSA from the circulation of the recipients was characteristic of an anamnestic response. The authors calculated that the total homologous antibody synthesized by the transferred cells during the first eight days of the secondary response amounted to approximately two-thirds of the wet weight of the transferred cells.

Incubation of lymph node cells with antigen *in vitro:* In experiments with lymph node cells from uninjected donors Harris and Harris[116] found that such cells could be incubated *in vitro* with dysentery bacilli, washed and transferred to irradiated recipients with the subsequent appearance of agglutinins to dysentery bacilli in the sera of the latter. Since it was estimated experimentally that about 4 to 5 per cent of the bacilli were carried into the recipient with the cell suspension, the possibility was considered that under these experimental conditions adequate contact between cell and antigenic material could have occurred in the recipient. In a later study[117] a soluble form of the antigen was used for *in vitro* incubation with the lymph nodes cell, with results similar to those described. It was obvious that with the use of a soluble form of the antigen a greater fraction of the antigenic material incubated with the cells would be removed from the cell suspension in the course of successive washings. (In the case of I*BGG and I*BSA, Roberts and Dixon[118] found that after lymph node cells had been incubated with these antigens and washed once, 1.8 per cent and 0.3 per cent, respectively, of the radioactivity remained in the cell suspension.) The finding of agglutinins in irradiated recipients after transfer of cells incubated *in vitro* with the soluble antigenic material, in the experiments of Harris et al. referred to,[117] suggested that some initial reaction between antigen and cells may have taken place in the test tube. This suggestion was supported by the observation that when the period of incubation between cell and antigen was less than thirty minutes at 37°C. the titers which appeared subsequently in the recipients were lower, and after incubation at 4°C. still lower.

In the work of Roberts and Dixon previously mentioned, lymph node cells from uninjected donor rabbits as well as from those injected with bovine γ-globulin or bovine serum albumin were incubated with these antigens *in vitro* and transferred to irradiated recipients. Antibodies to these antigens could not be detected in the sera of the recipients of such cells although antibodies were detectable if the irradiated recipients were also injected with I*BGG and I*BSA, as already mentioned.

Cytologic observations in the course of cell transfer studies: In some of the investigations of antibody formation by the transfer of cells of the lymphatic system, cytologic data have been reported. These are shown in Table 1. As can be seen these data were obtained under different experimental conditions, varying with the lymph node employed and the conditions of contact with the antigen. Certain similarities in the data are evident, in particular the preponderance of members of the lymphocytic series among all cells counted and, within this cell type, the preponderance of small lymphocytes. There are, however, differences in the percentages of various cell types reported. These may reflect to some extent the variations in experimental conditions and the differences in cytologic identification among the observers.

The evidence obtained in almost all of the studies involving cell transfer in relation to the formation of antibody has indicated that viable cells are necessary for the process to occur. Two hypotheses have been considered for the role of the transferred cells in the production of the

TABLE 1

PERCENTAGE DISTRIBUTIONS OF CELL TYPES IN SMEARS OF CELL SUSPENSIONS IN STUDIES BY VARIOUS INVESTIGATORS INVOLVING THE TRANSFER OF LYMPH NODE CELLS

	Chase[102]	Rebuck et al.[119,120]		Mitchison[113]	Harris et al.[121]		Roberts, Dixon[118]	
Antigen injected:	Picryl chloride	Bacterial cells, erythrocytes		Tumor cells	.....		Serum proteins	
Contact of cells with antigen:	In donor animal	In donor animal		In donor animal	*In vitro*		Both	
Source of cells:	Spleen and lymph nodes	Popliteal lymph node		Pooled lymph nodes	Popliteal lymph nodes		Pooled lymph nodes	
Cells	Per cent	Per cent		Per cent	Per cent		Per cent	
		Range	Average		Range	Average	Range	Average
Lymphocyte series:								
Small	....	52.8–84.2	72.4	....	87.5–97	92.6	57–84	70
Medium	....	7.8–28.4	14.3	....	.....	....	.....	..
Large	....	3.2–15.9	7.1	....	.....	....	5–24	15
Prolymphocyte	....	0.3– 2.3	1.2	....	2–9.5	5.4	.....	..
Lymphoblast	....	0.1– 1.0	0.3	....	0–2	1.0	.....	..
Total	≥ 95	85.2–98.0	95.3	>95	97.5–100	99	82–91	85
Plasmacytes:								
Total	....	0.5– 3.9	1.9	....	0– 1.5	0.6	0.5–5	3
Other	≤ 5	.....	2.8	<5	.....	0.4	.....	12

antibody found in the serum of the recipient: that the transferred cells are directly involved in the synthesis of the antibody, or that they contribute to the formation of those antibodies by supplying some substance which can be used by the tissues of the recipient animal for such synthesis. If the transferred cells are directly involved in the synthesis of antibody, it would be reasonable to consider that the predominating cell type, the lymphocyte, which has been found in as high as 99 per cent of transferred cell suspensions, might be the cell involved in this synthesis, unless some evidence would point to one of the minority cell types present as the source of the antibody found in the recipient. Data relevant to this question have not been reported. There are two considerations which would make it less plausible to attribute the antibody produced in these experiments to one of the minority cell types. First, in the cell populations reported by Rebuck et al.[120] there was variation over a substantial range in the percentage occurrence of each of the minority cell types, e.g. eightfold in the case of the plasmacytic series, as shown in Table 1, without corresponding differences in antibody titers of recipients of the cell suspensions involved. Second, in the only study thus far which included quantitative estimation of antibody produced by the transferred cells Roberts and Dixon[118] calculated that the total of such antibody was approximately two-thirds of the wet weight of the cells transferred. Antibody production by any of the minority cell types would imply the production by these cells of several times their weight in antibody (in the case of the plasma cell, twenty times their weight).

CONCLUDING REMARKS

A considerable number of experimental studies have contributed to our knowledge or understanding of the cellular response which follows the contact of antibody-forming tissues with antigenic material, and of cellular sources

of antibody. Despite this large volume of data it is at present impossible to state which cell or cells synthesize antibody, whether one or more cell types are involved, and whether the same or different cells are involved in the respective organs and under various conditions of antigenic stimulation. The recent literature is largely concerned with either the lymphocyte or the plasma cell, and acceptance of the greater part of the data in this literature would necessarily imply that both of these cell types may be involved in some manner in the formation of antibodies.

In any attempt at present to formulate a theory providing for participation by both cell types in the synthesis of antibody one would face several logical difficulties. First, there are disagreements among cytologists and other workers in this field as to the characterization and interrelations of the cells themselves. Thus the "acute splenic tumor" cell which was called a lymphoblast by Rich et al.[66] is probably the one named a transitional cell by Fagraeus,[71] and an immature plasma cell by Kolouch et al.[122] Again, the relationship between the lymphocytic and plasmacytic series of cells is not clear, both with regard to cytogenesis and to interchanging cell lines. Thus suggestions can be found in the literature that a common stem cell might function as precursor of both series of cells,[123] or that the immature plasma cell may develop from the immature lymphocyte.[72]

Second, there are uncertainties as to the extent to which experimental conditions affect the cellular response, or even the cellular source of antibody. It is not known how significant in this regard are such differences as those between single and multiple injections of antigens, grades of severity of the antigenic stimulus, forms of antigenic material (whether cellular and soluble), sites of injections and their relationship to the tissue studied, etc. It may be noted, for example, that the importance of selecting for study tissue which is regional to the site of injection of antigen has received increasing recognition in the more recent literature.[79,109]

Because of the wide variation in experimental conditions and cytologic emphasis in the studies in this field, the total mass of data available thus far does not lend itself to unifying generalizations. It may be useful, however, to recall the types of experimental data which would seem to stand in strongest support of the plasma cell or of the lymphocyte, respectively. In the case of the plasma cell one might mention the following: (1) The finding by Bjørneboe et al.[70] that in rabbits intensively immunized with pneumococci extracts of the fat of the renal pelvis containing cellular infiltrations were higher in antibody content than were extracts of other tissues not similarly infiltrated. The population of the cellular infiltrations consisted of 90 per cent plasma cells and 10 per cent lymphocytes. (2) The observation by Fagraeus[75] that antibody was produced *in vitro* by fragments of red pulp, but not of white pulp, obtained from spleens of animals given secondary injections of bacterial antigens. (3) The finding by Coons et al.[90,91] that fluorescence-stained antibody was associated with groups of plasma cells in the lymph nodes and spleens of animals, following secondary injections of antigens.

As to the relation of the lymphocyte to antibody formation, the data which would appear to lend strongest support are: (1) The finding, in lymph emerging from lymph nodes draining sites of single injections of antigens, of higher antibody titers in cell sediments than in the lymph plasma. Of the cells extracted in such experiments 99 per cent were found to be lymphocytes.[67,74] (2) The finding by Wesslén[78] of *in vitro* production of antibody by cells from the thoracic duct lymph of animals injected twice with bacterial antigen. No cells other than lymphocytes were found in these suspensions, nor was there evidence of change of cell type during incubation. (3) The strong preponderance of lymphocytes among the suspensions of cells used in recent cell transfer studies. Of the investigations by this technic in which results of differential cell counts were indicated, an average lymphocyte percentage of over 95 was found in two studies,[102,113] and in three others the average lymphocyte percentages have been found to be 85, 95 and 99, respectively.[118,120,121]

There is, then, a body of experimental evidence in favor of antibody production by each of these cell types. The acceptance of these data could imply, as one possible conclusion, that the two cell types may have parallel roles in the formation of antibody. On the other hand the two cell types may be related, either through a common precursor or by interconversion, and each may be operative under different conditions of contact between mammalian tissue and antigenic substances or in different stages of the synthesis of antibody. Although suggestions consistent with the latter concept are implicit in

some of the current literature, the data which would clearly establish it are lacking. It is likely that a considerable amount of clarification by experimental data will be required in order to approach an understanding of the role of each of these cell types in the production of antibodies.

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Clinico-pathologic Conference

Acromegaly, Diabetes, Hypermetabolism, Proteinuria and Heart Failure

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A FORTY-SIX year old white married housewife (No. 111445) was admitted to the Barnes Hospital for the first time on February 7, 1944. The patient dated the onset of her difficulties at the age of eleven when she began menstruating. Her periods were always irregular and associated with marked dysmenorrhea. At age twenty she married; and although contraceptives were not used, she never became pregnant. When she was twenty-four, uterine curettement was performed for dysmenorrhea and menorrhagia; these symptoms remained unchanged. At the age of twenty-five she underwent subtotal hysterectomy and removal of all ovarian tissue except a small portion of one ovary. During the ensuing year the cervix and remnant of ovary grew tremendously and another operation had to be performed to remove them.

In 1925, at the age of twenty-seven, the patient began to experience severe headaches and noticed that she could not "bite a thread" anymore. Her father noted that her mandible had increased in size. At this time she was told by a physician that she had acromegaly. She stated that shortly after the diagnosis was made "shot gun vision" developed which was confirmed by examination of visual fields by an optometrist. The patient was given a total of 772 roentgens to the pituitary through bitemporal ports, following which headaches were relieved and vision returned to normal. The patient stated that her jaw seemed to stop growing but that her feet and hands continued to grow (in 1938 her hands and feet stopped growing and her jaw began to enlarge). In 1930 she was admitted to another hospital where she was told she did not have diabetes. In 1940 she was admitted to still another hospital where she was told she did have diabetes, but refused insulin. She was placed on a dietary regimen which she did not follow. Her appetite was moderate but she complained of being thirsty all the time. The patient stated that during her entire adult life she had had progressive decrease in energy but not a decrease in libido. In 1944 she was started on 25 units of protamine zinc insulin each day. However, during the ensuing years her insulin requirement progressively increased so that by 1949 she was taking a daily total of 120 units of insulin, protamine and regular mixed, and in 1950 she took as much as 160 units a day; in subsequent years her requirement fell to approximately 80 units of insulin each day. She did not adhere to a diabetic diet and for the most part had 4 plus glycosuria (in spite of a high renal threshold for glucose), polyuria and polydipsia. Her weight remained reasonably stable, at approximately 200 pounds. In 1950 she was found to have advanced diabetic retinopathy with multiple capillary aneurysms, small deep hemorrhages and one flame-shaped hemorrhage on the right.

The patient's cardiac status was one of progressive but gradual deterioration. In 1949 she had her first episode of frank cardiac decompensation, which began with dyspnea on moderate exertion and progressed very rapidly to orthopnea, paroxysmal nocturnal dyspnea, ankle edema and cough productive of blood-tinged sputum. Digitalis was administered without much improvement. The patient then received six injections of a mercurial diuretic with resultant diuresis and regression of symptoms. She never adhered to a low salt diet. Administration of digitalis was continued until her demise.

In 1951 she was hospitalized at an outside hospital for an episode of severe pain in the back and chest; she was told she had had a "heart attack." Because of chronic cardiac insufficiency which she manifested the remainder of her life the patient lived an essentially sedentary existence, requiring weekly mercurial injections. Her blood pressure was always within normal range except on one admission in 1953 when it was recorded as 165/95.

In 1946 the patient began having episodes lasting three to six months each during which time her appetite increased, she became intolerant of heat, and lost weight. These symptoms seemed to abate when she was given small doses of propylthiouracil and iodine. Following the development of cardiac insufficiency it was observed that these symptoms were accentuated during the episodes of apparent increased metabolic activity. In 1950 the patient's radioactive iodine uptake was found to be 21.7 per cent. She was given 15 mc. of radioactive iodine. In 1952 the patient's radioactive iodine uptake was 46 per cent. Seven months later she was given 10 mc. of radioactive iodine. In the early part of 1951 she was given a total of 2,300 roentgens in air through each of three separate ports directed at her pituitary gland area.

Family History. Two cousins were known to have diabetes mellitus. Her paternal grandmother was known as "Big Kate." The family history was otherwise non-contributory.

Eighth Hospital Admission (August 6 to 9, 1954). For the first five months following the seventh hospital admission the patient's condition remained static until three months prior to this final admission, when she found that diuresis no longer occurred following injections of mercurial diuretics. Thereafter she noted progressively increasing dyspnea, orthopnea and ankle edema. Three weeks prior to admission she had an episode of substernal pain with radiation to the left shoulder and down the left arm. This pain lasted about two hours and subsided spontaneously. For one week prior to admission the patient had noted irregularity of her heart beat.

Physical examination on August 6th revealed a temperature of 37°c., pulse 120 and blood pressure 150/70. The patient presented a striking picture of marked enlargement of the skull, hands and feet with extreme enlargement of the mandible and bosselation of the frontal bones. She was extremely weak and required assistance to move about the room. She appeared drowsy and manifested some impairment of memory for both recent and past events. Her skin was thick and coarse. There was no lymphadenopathy. Examination of the eyes revealed constricted visual fields as determined by confrontation. The pupils were round and equal and reacted to light. Funduscopy revealed arteriolar narrowing, increased light reflex, old and fresh punctate and flame-shaped hemorrhages without papilledema. The palate was arched and the gums hypertrophied. The tongue was extremely large and fissured. The thyroid gland was palpable and there was no venous distention in the neck. Examination of the lungs revealed moist rales over the lower half of each lung field posteriorly. Borders of the heart were obscured. There was a grade 2 apical systolic murmur. The rate was grossly irregular. The liver was percussed three fingerbreadths below the right costal margin and the edge was not palpable; there was marked kyphoscoliosis. There was 2 plus pitting edema of the feet and legs extending up to the knees. The anal sphincter was relaxed; the external genitalia were hypertrophied. The deep tension reflexes were absent. The remainder of the physical examination was not remarkable.

The patient was given 40 units of protamine zinc insulin and 80 units of regular insulin mixed in the same syringe each morning. Fractional urinalyses gave consistently negative results for sugar. She was maintained on a diabetic diet and digitalis. Mercuhydrin® in doses of 2 cc. was given intramuscularly with no diuretic response. Because of dyspnea nasal oxygen was started on the second hospital day. On the third hospital day the heart rate had decreased from a high of 160 to 140 and was still irregular. On the morning of the fourth hospital day the patient was noted to be extremely dyspneic, with an apical heart rate of 175. Later that day she was found unconscious, pulseless and without obtainable blood pressure.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: Because we thought that a purely chronologic summary of this patient's extensive hospital records would be confusing, we have summarized briefly first the diabetic and acromegalic history, second cardiac complications, third hypermetabolism and fourth a discussion of the final hospital admission. We should like to begin this conference by showing some pictures of this patient at various stages during her life. We are indebted to Dr. Harold

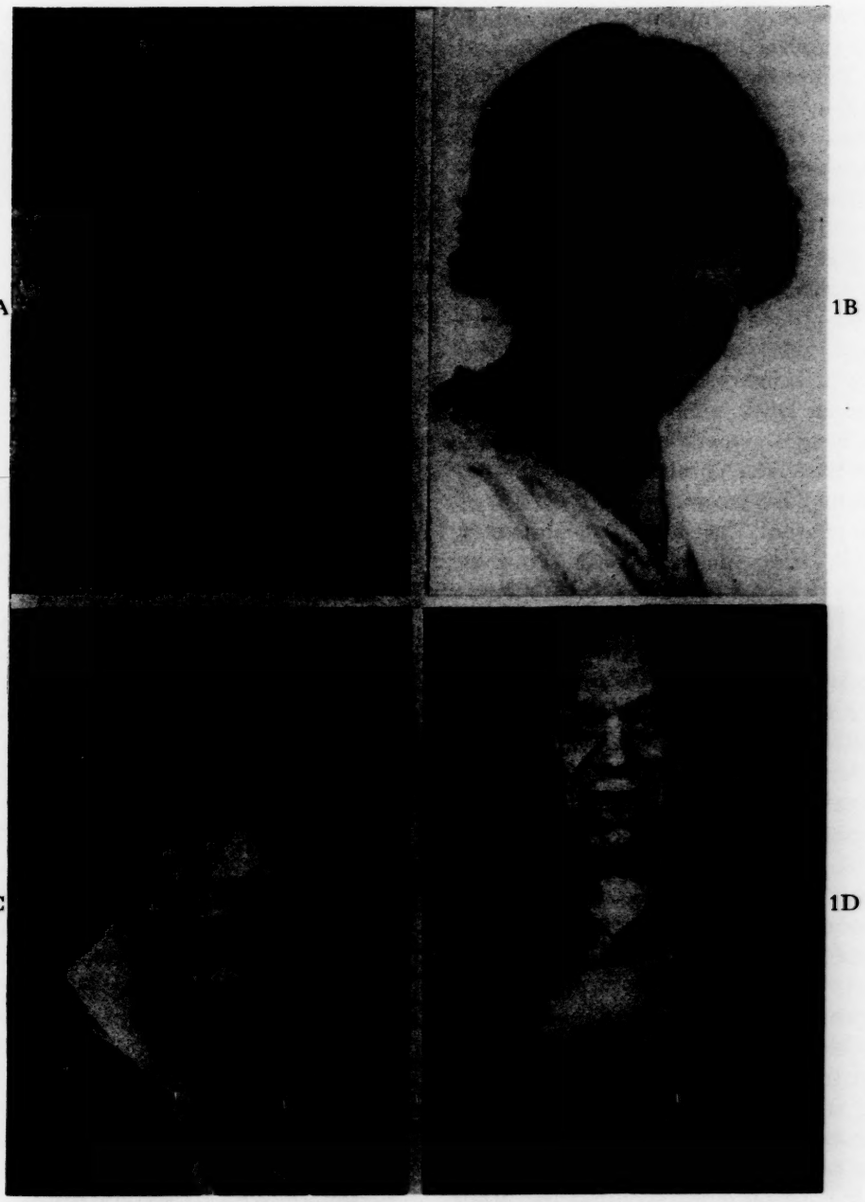

FIG. 1. Patient N. M. H. A, age nine years; B, age sixteen years; C, age thirty-three years; D, age fifty-two years.

Joseph for securing these photographs from the patient's husband. Figure 1A is a photograph taken in 1907. At this time the patient was a very attractive nine year old girl who appeared perfectly normal. Figure 1B shows the patient in 1914 at age sixteen. The jaw is perhaps a bit more prominent than is normally seen, but not strikingly so. Figure 1C is a picture of the patient in 1931 at age thirty-three. The face at this time has become quite characteristic of acromegaly. Note the enlargement of the lower jaw, thickening of the lips and enlargement of the nose as compared to the previous photograph. Nobody would hesitate in diagnosing the lady in this photograph as having acromegaly. Figure 1D shows the patient in 1950 at age fifty-two, four years before her death. This photograph was not posed by a portrait photographer as was the previous one, but rather by Mr. Cramer Lewis of our Illustration Department, especially to bring out the abnormal features. We have an opportunity here to see the hands for the first

time and can note the prominent frontal bossing, the large nose, the very thick lips, the marked prognathism. There is no question regarding diagnosis; you can see a patient like this 50 feet away and know that that this is a case of eosinophilic tumor of the pituitary with acromegaly, and there is no laboratory test that can disprove this.

Dr. Daughaday, in discussing this case we will constantly be referring to the pituitary hormones. In order to get this discussion oriented properly, I wish you would list the currently accepted pituitary hormones.

Dr. William H. Daughaday: Workers in the field generally accept growth hormone, corticotrophin, thyrotrophin, luteinizing hormone, follicle-stimulating hormone and prolactin as established hormonal fractions of pituitary extracts. The diabetogenic activity of pituitary extracts probably can be explained on the basis of their growth hormone content, along with the synergistic effect of smaller amounts of ACTH and prolactin. It is too early to predict whether other activities attributed to pituitary extracts such as the "R.Q.-lowering factor" studied by Recant and others, the fat-mobilizing factor, the hematopoietic-stimulating factor and a number of others will prove to be independent pituitary hormones.

Dr. Reinhard: Would you also, Dr. Daughaday, give your opinion of the pituitary cell of origin of these hormones which you have mentioned?

Dr. Daughaday: As this question involves the interpretation of cytologic evidence, I can only discuss it from the point of view of an outsider. Re-examining the rat pituitary with newer methods, Purves, Griesbach and others have described two types of basophils, one of which is related to thyrotrophin activity and the other to gonadotrophin activity. The origin of ACTH is much debated. The work of Finnerty, formerly of this school, suggested that ACTH was produced by eosinophils. Clinical evidence, however, in Cushing's disease and Addison's disease would indicate that this hormone arises from the basophils. The pathologic evidence in acromegaly leads to the conclusion that the eosinophils produce growth hormone. It is possible that prolactin is also a produce of the eosinophils.

Dr. Seymour Reichlin: Dr. Reinhard, an experiment using an antibody method for localizing ACTH was reported in a paper by Marshall in 1951. Rabbits were prepared with ACTH in such a way as to form ACTH antibodies, which were labelled with fluorescein; sections of anterior pituitary were treated with these fluorescein antibodies. There was no question that ACTH seemed to be produced in basophils. ACTH is the only pituitary hormone, probably, which does not stain; and if you base your deductions on staining reactions, the only hormones that the acidophils make would be the growth hormone and the lactogenic hormone.

Dr. Reinhard: Thank you very much, Dr. Reichlin. Dr. Seaman will comment on some x-rays, selected from the tremendous number made on this patient.

Dr. William B. Seaman: The lateral view of her skull taken in 1950 showed typical acromegalic changes in the large protruding mandible, tremendous enlargement of the frontal sinus, and extensive pneumonitization of the mastoid area. The sella turcica is enlarged in both the depth and the anteroposterior diameter, the dorsum being intact, which is the usual case in eosinophilic tumors. The remainder of the skull was seen to possess marked thickening, particularly in the frontal bone near the vertex. Examination of the chest in October, 1949, demonstrated marked cardiac enlargement, predominantly involving the left ventricle; the lung fields showed some dilatation and engorgement, with vascular markings suggesting pulmonary hypertension. The diaphragms were of equal height in this examination, but as we followed the leaf of the left diaphragm during 1952 and 1953, particularly, we noticed a progressive rise in its height. In the lateral view this was particularly prominent, occupying the posterior half of the large gastric air bubble. This was the finding which suggested that the patient might possibly have a diaphragmatic hernia; however, two gastrointestinal examinations done in 1950 and 1952 failed to confirm the presence of diaphragmatic hernia. This left leaf of the diaphragm had nearly normal movement on fluoroscopy and probably was partially paralyzed. The remainder of the chest films showed some evidence of progressive cardiac enlargement and progressive increase in the amount of pulmonary congestion. Another interesting finding which I found difficult to interpret was some widening of the superior mediastinum seen in all these films, which progressively increased. It may have been due to dilatation and elongation of the large vessels arising off the aortic arch, but we could

not exclude the possibility that there could also have been a substernal enlarged thyroid in this area. The skeletal system was notable for some enlargement of the vertebrae, usually seen in acromegaly, with moderate hypertrophic changes.

DR. REINHARD: Dr. Seaman, this patient was given 772 roentgens to the pituitary at age twenty-seven with, it was stated, relief of headaches and return of normal vision. She was having visual disturbances, and this was a very small dose of x-ray. I wonder if you would comment on the usual radiosensitivity of these tumors. Is it not rather surprising that she had so much improvement from this small dose?

DR. SEAMAN: That dose was even smaller than it now seems because it was actually 386 roentgens to each side, which represents a dose to the pituitary probably in the range of 200 and 250 roentgens. I certainly am surprised that she noticed any regression of symptoms, although it is said that some of these tumors are markedly radiosensitive; however, present opinion is that the best results are obtained with doses at the level of pituitary which range from 3,000 to 3,500 roentgens.

DR. REINHARD: Is it not true that the normal pituitary is quite resistant to radiation effects?

DR. SEAMAN: The normal pituitary is extremely resistant. Some experimental work has been done on patients with advanced carcinoma of the breast in which an attempt was made to destroy the normal pituitary; doses as high as 14,000 roentgens were given without any demonstrable clinical effect on the pituitary.

DR. REINHARD: We have followed here one of Dr. Howard Bierman's patients who was treated for advanced carcinoma of the breast with 9,000 roentgens to the pituitary. Until she died three years later, she never showed any clinical manifestations of hypopituitarism, and at autopsy showed no pituitary abnormalities

Dr. Sherry, in acromegaly other striking changes sometimes occur in the soft tissues, connective tissues, tendons, muscles and skin. This patient had typical thick acromegalic skin. She had a tremendously enlarged heart and probably hypertrophy of the heart muscle and other muscles as well. Would you tell us what is known about the effect of the endocrine disturbances in acromegaly upon the connective tissue and collagen?

DR. SOL SHERRY: Before attempting to answer the question specifically, I think it would be important for us to orient ourselves with respect to the biochemistry of connective tissue in general. The chemist has identified fat, collagen and at least six different acid mucopolysaccharides as constituents of connective tissue. Collagen, which is the major constituent, is an insoluble fibrous protein which is very resistant to attack by all of the usual proteolytic enzymes. It has a rather characteristic appearance when viewed under the electron microscope, and a very unique amino acid composition. It contains about 15 per cent hydroxyproline, an amino acid which seems limited in the whole animal kingdom to collagen. In addition there are very large amounts of glycine and of proline; about 40 to 50 per cent of the collagen is made up of glycine, proline and hydroxyproline. At present, our methods for studying collagen in disease states are rather limited to such things as the appearance in electron photomicrographs and the hydroxyproline analysis of collagen extracted from tissue. With regard to the acid mucopolysaccharides, two of them are known to be very viscous. One is the very well known hyaluronic acid, and more recently chondroitin has been isolated. Both of these materials are depolymerized or broken down by hyaluronidase. The other four acid mucopolysaccharides are sulfated. There are three chondroitin sulfates, a, b and c, and very recently a kerato-sulfate has also been obtained. The physical nature of these materials in connective tissue is still to be determined. At present, methods for studying these materials are even much cruder than those of studying collagen, and we are limited to a staining of the acid mucopolysaccharides by toluidine blue or the periodic acid-Schiff reaction and other similar stains. In addition, hyaluronidase has the ability to increase spreading of fluids through the connective tissue. Now, specifically, in acromegaly it is known that there is a general increase in the connective tissue throughout the body. In the few studies that have been carried out it would appear that there is a considerable quantitative increase in collagen. Whether or not the collagen that has been isolated has a normal appearance in electron photomicrographs and a normal content of hydroxyproline, I do not know. In addition, the collagen is present in a normal ratio to the amount of water in the tissue. Insofar as the acid mucopolysaccharide content is concerned, by staining reactions it appears that there is a normal amount of these materials, and

that there is no metachromasia seen with toluidine blue; however, upon injection of hyaluronidase into the skin of an acromegalic, it has been shown that there is a very definite delay in spreading. Whether this is due to some qualitative abnormality in the polysaccharides or to impediment from the increased amount of collagen fibers present remains to be determined. From these few studies, it would seem that in summary, there is a very marked increase in collagen *per se*, which would be consistent with increased amounts of protein synthesis under the influence of the growth hormone.

DR. REINHARD: One of this patient's earliest symptoms was dysmenorrhea, plus menstrual irregularity and sterility. She was married at the age of twenty, and in spite of the fact that contraceptives were not used, she never became pregnant. At age twenty-five hysterectomy was performed with removal of all of one ovary and most of the other ovary. During the following year there was tremendous growth and hypertrophy of the remaining fragment of ovary and another operation was performed on the stump of the cervix which was also hypertrophied; both were removed. A year or so after the second operation severe headaches developed and enlargement of the jaw characteristic of acromegaly was noted. Dr. Woolf, is there any relationship between the removal of the ovaries and acromegaly?

DR. RALPH G. WOOLF: In this particular case one is very suspicious of a person who is said to have had irregular menstrual periods and dysmenorrhea. Dysmenorrhea *per se* is usually associated only with ovulatory cycles. There are certain specific castration changes that occur in the pituitary gland when the gonads are removed. This usually consists of hypertrophy of the basophilic portion of the pituitary gland, or at least that element containing basophilic cells.

DR. REINHARD: Chronologically, the next clinical problem that arose in this patient was diabetes. Dr. Recant, considerable work has been done in our own biochemistry department on the effects of the pituitary substances on carbohydrate metabolism; would you discuss the present state of our knowledge regarding the influence of pituitary hormones on carbohydrate metabolism?

DR. LILLIAN RECANT: If I may, I would like first to make one comment on estrogens and acromegaly. Estrogen has been used in the treatment of acromegalics, with very good results, so that actually one might anticipate that there could have been an effect of the removal of the gonads upon this process going on in the pituitary. Secondly, there have been many reports that patients with acromegaly have become pregnant and had normal deliveries.

Coming back then to the question you asked, the present status of the effects of pituitary hormones on glucose metabolism is a complicated problem. I think one can outline it, perhaps, in this way: It is known that adrenotrophic hormone is diabetogenic. It is known that the growth hormone is diabetogenic. It is also known that the thyrotrophic hormone can intensify existing diabetes. Therefore, it would appear that if one had hyperfunction of the pituitary, this might be contributing diabetogenic substances from any one of these three hormones, depending upon which cells were hyperfunctioning. In acromegaly *per se*, growth hormone production certainly is presumed to be increased, and this is probably related to the diabetic state. There has been very little clinical evidence that ACTH secretion is increased in acromegaly, but I believe that there have been cases reported of Cushing's syndrome in patients with acromegaly. With respect to the absolute effects of these hormones on glucose metabolism, ACTH is chiefly an insulin-antagonizing type of substance, and to some degree potentiates the diabetogenic effects of growth hormone in inhibiting glucose uptake by the peripheral tissues. Growth hormone is also presumed to be an insulin antagonist. It has been shown to inhibit the glucose uptake of tissues, to inhibit the conversion of C-2 fragments to fatty acids, and also to have an effect on the respiratory metabolism of the tissue, suggesting that it may have an action in addition to its effect on glucose uptake, namely, below the hexokinase level of the glycolytic cycle. As far as thyrotrophic hormone is concerned, the diabetogenic effects, I believe, are chiefly associated with the effect on the increase in metabolism, the diminished ability to store glycogen in the liver, and also the increased rate of absorption of glucose from the intestines.

DR. REINHARD: Dr. Daughaday, another possibility here would be that this patient may have had the ordinary form of hereditary diabetes mellitus. She had two cousins who had diabetes. I do not believe that "Big Kate" was one of these, but do you think this patient probably had disturbance of glucose metabolism

related entirely to her acromegaly, or was this independent diabetes mellitus?

DR. DAUGHADAY: That is a very difficult question to answer. When growth hormones are given to experimental animals, one of the very important factors determining whether diabetes is produced is the ability of the pancreas to produce insulin. Certain animals, such as the rat, apparently have a tremendous ability to produce insulin and it is very difficult to produce any alterations in the carbohydrate metabolism of the intact rat; however, if one removes three-quarters of the pancreas, or adds cortisone, and then administers a growth hormone, diabetes can be produced in this species. Therefore, in this battle between insulin and growth hormone not all patients with acromegaly become diabetic, presumably because they have a greater pancreatic reserve. I would imagine that any factors such as hereditary limitation of pancreatic reserve would precipitate diabetes of a more severe nature at an earlier time in such individuals.

DR. REINHARD: We may presume then that this patient had a hereditary diabetic tendency, and it was greatly aggravated and intensified, and brought on at an earlier age by acromegaly. Is that right?

DR. DAUGHADAY: It is a suggestion, at least.

DR. REINHARD: Would you also comment very briefly on the treatment of this patient's diabetes. She required very large doses of insulin, which is often the case in acromegalic diabetes.

DR. DAUGHADAY: Too much emphasis has been placed on insulin-resistant acromegalics, because statistically they are infrequent. In most patients with acromegaly, the abnormality in carbohydrate metabolism is purely a laboratory abnormality brought out by glucose tolerance tests. Only about 20 per cent of these patients are diabetics in a clinical sense, and I would say that the majority can be handled by insulin in the usual doses. The patient described by our group, who required up to 600 units a day, was exceptional, and any patient requiring more than 100 units daily would actually be an unusual acromegalic. The treatment of this patient for diabetes was difficult because she did not follow the prescribed regimen. She was insulin resistant, and in later years was also severely handicapped by cardiac disease.

DR. REINHARD: To continue in chronologic order, the next clinical problem that arose in this patient was the development of symptoms suggesting hypermetabolism. Starting about 1946 the patient began having episodes during which her appetite increased, she had heat intolerance and weight loss, and her symptoms seemed to abate when she was given small doses of propylthiouracil, thiouracil and iodine. Following development of cardiac decompensation, it was observed that symptoms of cardiac insufficiency were greatly accentuated during times when she had apparent hypermetabolism. Her basal metabolic rate was always high. In 1950 her radioactive iodine uptake was found to be 21 per cent. She was given 15 mc. of radioactive iodine, presumably to help control cardiac decompensation. In 1952 her radioactive iodine uptake was found to be 46 per cent, at which time an additional 10 mc. of radioactive iodine were administered. Dr. Daughaday, would you discuss the relationship of acromegaly to hypermetabolism?

DR. DAUGHADAY: This is a question about which opinion has been changing in recent years, due to the use of protein-bound iodine and radioiodine technics. The older view was that all elevated basal metabolic rates in patients with acromegaly were due to an excessive production of TSH and resultant hyperthyroidism. Observations by McCullogh and others have shown that in many patients with acromegaly there seems to be normal thyroid function, so that it would appear that there is an actual direct effect of the pituitary on oxygen metabolism. The mechanism is still obscure. The first observations made on this patient were entirely consistent with this view, but on subsequent observation, a definite increase in the radioiodine uptake would make one think that true hyperthyroidism may have developed in this patient.

DR. REINHARD: This patient did have a goiter, did she not?

DR. DAUGHADAY: Yes. Goiter is present in 40 per cent of patients with acromegaly; it is a nodular goiter, the activity of which is difficult to assess on histologic analysis.

DR. REINHARD: The next problem has to do with the patient's cardiac decompensation and heart disease, a very important part of this patient's story. In 1949 she had her first episode of frank cardiac decompensation and during subsequent years she had constant trouble with chronic decompensation requiring mercurial injections at frequent intervals over a long period

of time. She finally died in cardiac decompensation. Three weeks before her final admission to the hospital, about three and one-half weeks before her death, she had an attack of severe pain in the left arm, with substernal pain. Dr. Massie, would you give us your opinion as to what type of heart disease this patient had, and also what you think about the electrocardiogram?

DR. EDWARD MASSIE: This patient had the potentiality for developing many types of heart disease. She had acromegaly and thus could have had acromegalic heart disease, which, as I understand it, refers to the general increase in heart size effected by the growth hormone. In addition, the patient suffered from the necessity of pumping her circulating blood through a tremendously increased amount of tissue, requiring increased work of the heart; we must not forget the factor of thyrotoxicosis. The patient also had arteriosclerosis. Three weeks before her death she had an attack of what appeared to be coronary artery disease. Electrocardiograms first showed left ventricular enlargement and strain and continued through to her terminal episode showing the same thing, although there was a gradual worsening of the tracings. We cannot make an electrocardiographic diagnosis of a coronary thrombosis. If we were to use our clinical judgment, we would say that the patient did have coronary insufficiency, and I do not doubt that she will have at autopsy either infarction or myocardial fibrosis or necrosis.

DR. JOHN SMITH: Acromegalic heart disease is characterized by massive hypertrophy of the myocardial fibers which is apparently not necessarily just a work hypertrophy, because they become enormously enlarged, even when low or normal blood pressures are present.

DR. ADOLPH SURTSHIN: Cardiomegaly may not be associated with any increase in systemic pressure, but rather with a rise in the cardiac output, which in turn is largely based upon an increase in the renal blood flow. There is a measurable increase in renal blood flow in these patients, at least in those reports with which I am acquainted.

DR. REINHARD: Let us mention briefly the renal lesion. This patient had long-standing proteinuria, and she had azotemia at the time of death. She certainly could have had Kimmelsteil-Wilson's disease. The patient had generalized arteriosclerosis and probably had considerable nephrosclerosis. I do not believe we can really go much farther than this. The final diagnoses I would like to present are acromegaly due to an eosinophilic tumor of the pituitary, diabetes mellitus, with hyperthyroidism in 1950 treated with I-131, acromegalic heart disease plus generalized arteriosclerosis, coronary arteriosclerosis, nephrosclerosis and, possibly, Kimmelstiel-Wilson's disease.

PATHOLOGIC DISCUSSION

DR. MALCOLM MCGAVRAN: At autopsy the body of this elderly white woman weighed 111.5 kg., approximately 240 pounds, and measured 170 cm. in length. The face was characterized by supraciliary hyperostoses, enlargement of the nasal cartilages, prognathism, thickened skin and moderate hypertrichosis over the chin and upper lip. Hands and feet were remarkably large in both width and length. Knees and elbows were also very prominent. The thoracic cage was markedly increased in all diameters and the ribs were wide and thin. This patient had a truly barrel-shaped chest. There was moderate kyphoscoliosis of the dorsolumbar spine and softening of the trabeculae within the vertebral bodies. The escutcheon was masculinized and hypertrichosis was present over the anterior thorax and thighs. Seven hundred ml. of clear serous fluid were present in the right pleural cavity, 900 ml. of a similar fluid in the left and 300 ml. of fluid in the peritoneum. Together the lungs weighed 1,200 gm. They were moderately congested and emphysematous at their margins. Evidence of primary tuberculous infection was found. The liver was large (2,490 gm.) and congested. The stomach was dilated, containing approximately 1 L. of gray fluid. Mucosa of the small bowel was congested, while that of the cecum and ascending colon was jet black, grading off to black-brown and brown in the transverse and descending portions of the colon. The uterus and the ovaries were absent. The heart was remarkably enlarged (1,020 gm.) in all its chambers, the walls of both left and right ventricles being more than twice their normal thickness. Both atria were also enlarged. Coronary arteries were enlarged and severely sclerotic, but neither old nor recent occlusion could be found. In the anterior portion of the intraventricular septum there was an area of fibrosis approximately 3 by 4 cm. in size. The anterior leaflet of the mitral valve and the

endocardium of the left ventricle were diffusely thickened. The kidneys were symmetrically enlarged, the right weighing 500 gm., the left 370 gm., but otherwise appeared relatively normal throughout both capsular and cut surfaces. The thyroid was large (220 gm.). It lay mostly within the superior mediastinum, only one-third reaching above the clavicle. It contained many firm, well encapsulated, brown nodules (1 to 3 cm. in diameter), many of which were calcified. The skull was thick (2 cm. at the vertex) and the frontal bone was moderately hyperostotic. The sella turcica measured 2.8 by 1.3 by 3 cm. containing an enlarged pituitary that weighed 5.5 gm. (normal weight 400 mg.). On cut section it consisted of a mass of soft, gray-white, slightly granular tissue. A thin rim of compressed white firm tissue surrounded the infundibulum at the superior pole.

Generalized visceromegaly was a prominent feature in this case. The thyroid was many times the normal size; the heart was more than doubled in weight, as were also the spleen and kidneys. In summary then, this case presented many but not all of the classic findings of advanced acromegaly, with visceromegaly (liver, kidneys, spleen, thyroid, heart), generalized arteriosclerosis particularly of the coronary arteries and signs of congestive failure.

DR. W. STANLEY HARTROFT: In microsections of the pituitary, the adenoma of the pars anterior observed grossly is made up almost entirely of eosinophils. (Figs. 2 and 3.) Only a few cells do not contain the characteristic granules, and even they appear to represent degranulated eosinophils. Around vessels, the cells frequently assume pseudorosette arrangements. This tumor, which is relatively vascular, exhibits all the classic features of a true eosinophilic adenoma of the pars anterior of the pituitary.

Islets in the sections of pancreas are larger and more numerous than normal and, as a result, the islet-acinar ratio is evidently increased. The beta cells are hypergranular (Fig. 5) and several estimations of the ratio of alpha to beta cells proved this to lie in the range of one alpha cell to every eight or ten beta cells, a ratio considerably less than that usually encountered at this patient's age.

Microscopically, interstitial tissue of the myocardium is abnormally abundant and the rather isolated muscle cells are enlarged. Outlines of their nuclei in sections are frequently square, indicating hypertrophy. The thyroid under the microscope presented the usual appearance characteristic of multinodular colloid goiter. The kidneys appeared as normal microscopically as they had grossly, but measurements of the glomeruli in section established that they were twice the normal diameter.* (Figs. 6 and 7.) Examination of multiple sections of kidney failed to reveal evidence of more than a slight degree of nephrosclerosis. Centrolobular parenchymal atrophy in the liver (Fig. 4) reflected the periods of cardiac decompensation, noted clinically.

Final Anatomic Diagnoses: Eosinophilic adenoma of the anterior hypophysis, 5,500 mg. (acromegaly—clinical history for thirty years); multinodular colloid goiter, 220 gm.; hypertrophy of islets of Langerhans (with hypergranulation of β cells) (diabetes mellitus, history for fourteen years); splanchnomegaly involving both kidneys (500 gm. and 370 gm.), liver (2,490 gm.), heart (1,020 gm); myocardial fibrosis (wall of left ventricle and septum); cardiac dilatation; hydrothorax (1,600 ml.) hydropericardium (160 ml.), ascites (300 ml.); congestive splenomegaly (520 gm.); chronic passive congestion, lungs and liver; macroglossia with fatty infiltration of muscle; generalized arteriosclerosis, particularly advanced in coronary arteries and abdominal aorta; arteriolar nephrosclerosis (slight); parathyroid hyperplasia; diffuse osteoporosis; kyphoscoliosis of dorsolumbar spine (advanced); hyperostosis frontalis interna; surgical absence of uterus, fallopian tubes and ovaries, vermiform appendix (linear abdominal scars) and melanosis coli.

The incidence of diabetes in acromegaly is reported in some series to be as high as 20 per cent.[1,2] In view of the diabetogenic action of growth hormone, it is perhaps surprising that the incidence in acromegalics is not higher. In these individuals, however, the islets of Langerhans may be able to respond abnormally well to increased demands for insulin. If true, unusually large amounts of insulin would be liberated, at least for a time, by the islets of the patients with acromegaly. Perhaps this sequence of events is an important factor in explaining the phenomenal growth which characterizes

* Performed by Dr. Malcolm McGavran.

[1] DARRAGH, J. H. and SHAW, W. M. Acromegaly and diabetes. *Canad. M. A. J.*, 64: 146–150, 1951.

[2] COGGESHALL, C. and ROOT, H. T. Acromegaly and diabetes mellitus. *Endocrinology*, 26: 1–25, 1940.

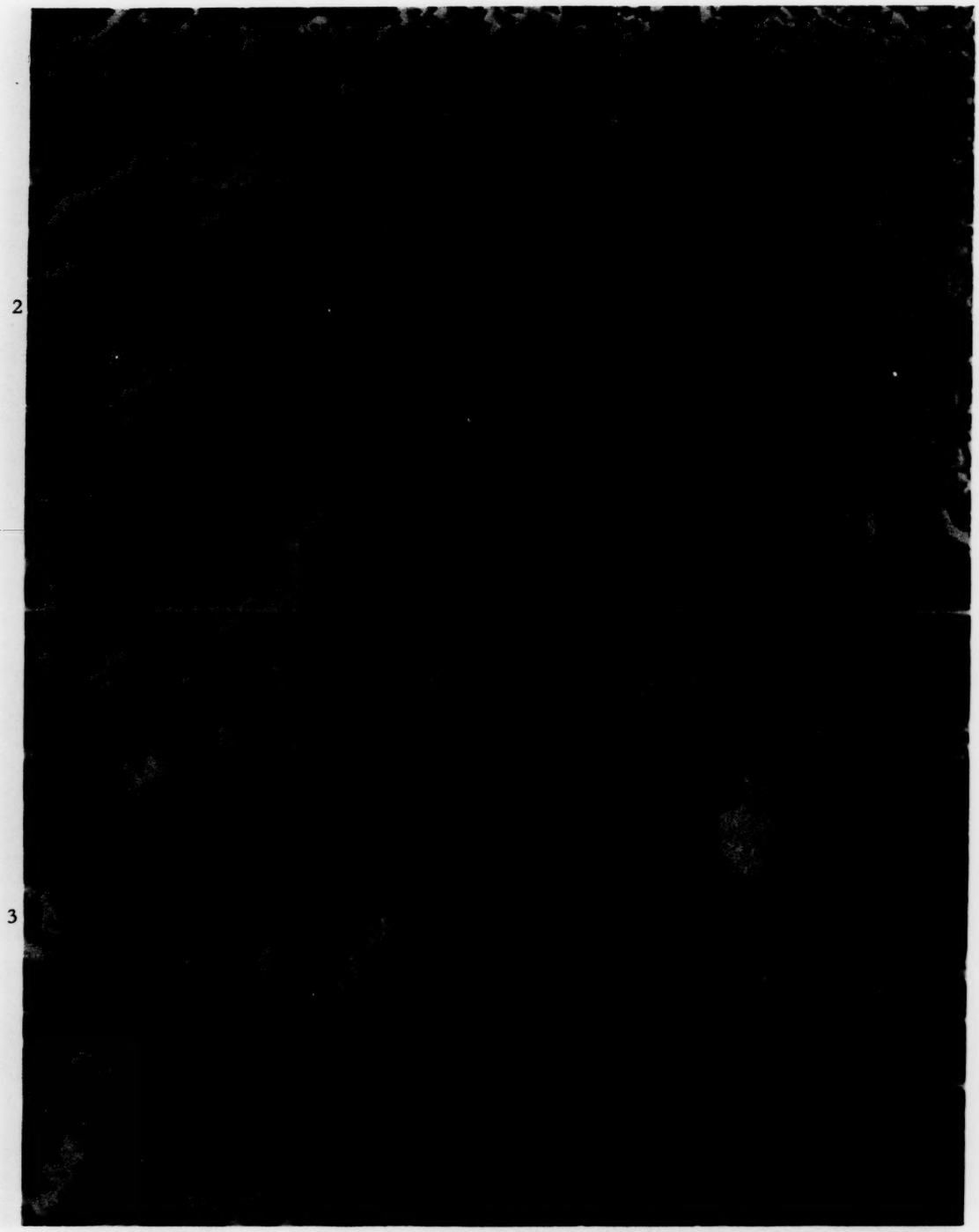

FIG. 2. Almost every cell in the pituitary adenoma contains granules which stained red with azocarmine (black in photograph). Compressed tissue at the edge of the tumor (upper right) is very vascular. Mallory's trichrome stain, photographed through Wratten B and G filters; × 300.

FIG. 3. Under higher magnification the cells in the tumor are frequently found arranged in pseudorosettes around small blood vessels. Same preparation as for Figure 2; × 900.

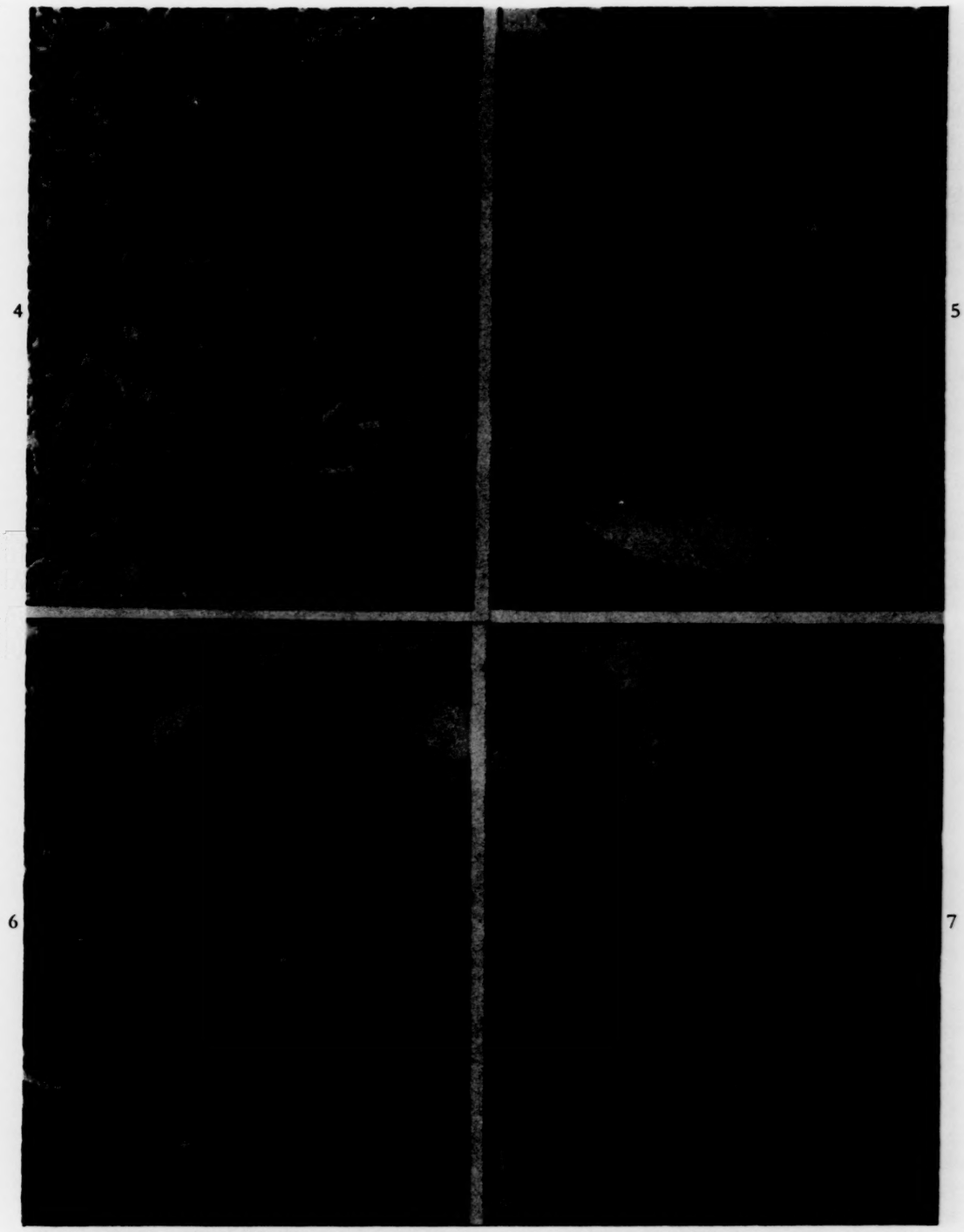

FIG. 4. Only those liver cells immediately around portal triads (lower right) had escaped degenerative changes secondary to chronic hepatic congestion. Many of the nuclei exhibit glycogenic vacuolation. Hematoxylin and eosin stain, photographed through Wratten B and G filters; × 100.

FIG. 5. Beta cells containing many granules which stained deep purple (black in photograph) with the aldehyde fuchsin technic greatly outnumber the occasional alpha cells which appear pale here. Wratten B and G filters; × 500.

FIGS. 6 and 7. This representative glomerulus (Fig. 6) in the subcapsular zone of the patient's renal cortex is almost twice the diameter of a comparable glomerulus (Fig. 7) in the same position in the kidney of a patient of same age, weight and sex. Both glomeruli have been cut in planes that include the afferent arteriole and the neck of the proximal convoluted tubule where it joins Bowman's capsule. Hematoxylin and eosin stain, photographed through Wratten B and G filters; × 150.

the condition. Insulin is a "growth hormone" *par excellence*, capable of producing all the manifestations of growth as variously defined, even in the absence of the anterior pituitary.[3] Extracts of the anterior pituitary are, however, incapable of producing sustained growth in diabetic animals. Growth to at least some degree may continue in the acromegalic patient only as long as the islets of Langerhans can respond with increased production of insulin to the excessive liberation of pituitary hormone. Diabetes in these patients would result only if even this high production of insulin could not fulfil the abnormally great need for this hormone created by the pituitary tumor. Growth would of course cease when the individual became frankly diabetic, for the diabetic individual cannot grow, a fact only too pitifully evident in children so afflicted before insulin became available.

In the case under discussion, insulin precurser in the form of beta cell granules is even more abundant than in normal individuals, indicating that complete "islet decompensation" had not yet developed, despite her clinical history. It is interesting to speculate what the course of her acromegalic disease might have been had diabetes developed earlier, a few years after the onset of her initial symptoms thirty years before death.

Acknowledgment: Clinical illustrations were made by the Department of Illustrations, Washington University School of Medicine. Photomicrographs were prepared in the Department of Pathology.

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Case Reports

Visceral Manifestations of American Mucocutaneous Leishmaniasis*

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CLASSICALLY, the term "leishmaniasis" has been used to include three distinct disease entities: Visceral leishmaniasis or kala-azar; cutaneous leishmaniasis or oriental sore; mucocutaneous leishmaniasis or espundia. Though clinically distinct, each of these is caused by a leishmania parasite which is transmitted by the bite of the Phlebotomus sandfly.

Visceral leishmaniasis is caused by Leishmania donovani and has been reported in both the Old World and South and Central America.[1] It is a chronic disease characterized by irregular fever, enlargement of the liver and spleen, anemia, leukopenia and hyperglobulinemia. The organism is found in the blood, bone marrow, liver and spleen. It is more frequent in children and if untreated has a mortality rate of over 90 per cent. Skin lesions are unusual and occur as a sequel to generalized infestation with the parasite.[2]

Cutaneous leishmaniasis is a specific ulcerating granuloma of the skin caused by L. tropica and has been reported in many warm countries. The disease is self-limiting and although there may be regional lymph node enlargement there is no alteration of the cellular and protein constituents of the blood.

Mucocutaneous leishmaniasis is caused by L. braziliensis. Its existence is, for the most part, limited to the Western Hemisphere, having been reported from nearly all parts of Central and South America, but it has been reported in other parts of the world from time to time. It occurs at any age and in either sex. The disease is characterized by the presence of cutaneous and mucous membrane involvement. There is usually a history of a primary lesion which heals, leaving a characteristic scar. After an interval of months or years the disease reappears and involves the mouth and nose. There may or may not be distal cutaneous ulcers, but almost uniformly the distal portion of the nares and nasal mucous membranes is involved in a granulating, ulcerating and sometimes mutilating process.[3] Ultimately the process may extend to involve the palate, pharynx and larynx and, if untreated, may lead to death.[2] The lymph nodes are often involved but it is generally agreed that the viscera are not involved.[2] It is the purpose here to present typical cases of American mucocutaneous leishmaniasis with evidence of visceral involvement, suggesting systemic disease with changes similar to but not as severe as kala-azar.

CASE REPORTS

CASE 1. A sixty-six year old colored man was referred to the Medical Service of Gorgas Hospital with a diagnosis of Hansen's disease. The patient had enjoyed good health until about one year before admission, at which time he had noted the appearance of several small nodules on the end of his nose. Six months later he began to experience a little difficulty in speaking and swallowing. These symptoms increased gradually in severity so that by the time of admission his voice was nearly inaudible and he could swallow only very soft foods. About four months before admission he had noted the onset of persistent hoarseness. The patient denied all symptoms of skin disorders with the exception of burning of the skin on his right shin due to local application of mustard for an injury there.

The patient was born in Jamaica and resided there for twenty-four years before moving to Port Simon, Costa Rica. At the age of fifty-two he moved to Bocas Del Toro, Republic of Panama. He remained there for two years and

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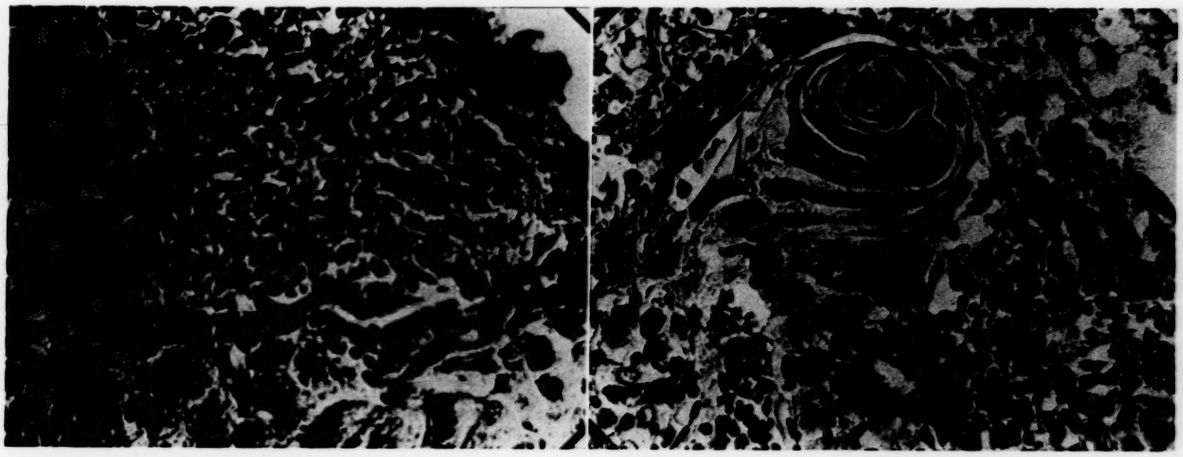
FIG. 1. Case I. Frontal view showing the ulcerative granulomatous lesion involving the nose and lip. The absence of the outer third of the eyebrows further suggested the erroneous diagnosis of leprosy for which the patient was originally hospitalized.

then, for the next eleven years, resided in the terminal cities of Panama and Colon, Republic of Panama. During this time his only sojourn in the interior of Panama was during World War II, while working for the U. S. Government. The past history and system review were not significant.

Physical examination revealed the following: Temperature 98.6°F., pulse 100, respiration 26, blood pressure 112/80, height 5 feet 6 inches, weight 112 pounds. This was a thin, poorly nourished colored man, alert and cooperative, who appeared chronically ill. The outer third of both eyebrows was missing. The pupils were equal and reacted to light and accommodation. Examination of the fundi revealed no abnormalities. There were many nodular and ulcerative lesions over the distal part of the nose. (Fig. 1.) These were predominantly about the external nares. On the vermilion border of the left upper lip was a 1 cm. oval ulcerative lesion with raised edges. All lesions were non-tender. The mucous membranes of the outer third of both nostrils showed granulomatous crusted lesions. The uvula and part of the left posterior region of the soft palate were absent. The remainder of the soft palate was covered by a granulomatous lesion. The upper third of the epiglottis was missing and the remainder of the epiglottis and both arytenoids showed thickening and granulomatous changes. The gag reflex was absent. Both vocal cords were thickened and injected, and there was a definite decrease in the size of the lumen in this area of the larynx. There was no stiffness of the neck. The lungs were clear to percussion and auscultation. The heart was not enlarged and no murmurs were audible. The abdomen was soft, and the tip of the liver and spleen were easily palpable on deep inspiration. Neurologic examination was essentially negative. There were soft submaxillary lymph nodes and several firm inguinal lymph nodes palpable bilaterally.

Laboratory findings were as follows: On admission the white blood count was 6,400 with 37 per cent neutrophils, 45 per cent lymphocytes, 12 per cent eosinophils and 2 per cent basophils. Routine urinalysis showed a trace of albumin and a few white cells per high power field. Red blood count was 3.9 million, hemoglobin 13 gm. Stool examination revealed ova of hookworm. Sedimentation rate was 32 mm./hr., hematocrit 34 per cent. Serologic tests for syphilis: routine flocculation test positive (with undiluted serum only), routine complement-fixation test negative. Bleeding time was three minutes, clotting time four minutes (capillary method). Icterus index was 5. Cephalin flocculation test was 3+. Serum bilirubin was 0.25 mg. per cent. van den Bergh test was negative. Prothrombin time was 100 per cent of normal. Bromsulphalein test showed 32 per cent retention at the end of forty-five minutes. The total protein was 8.4 gm. per cent with 2.9 albumin and 5.4 globulin. Serum non-protein nitrogen was 23. Heterophil and cold agglutination tests were negative.

Subsequent studies revealed the following: Scrapings from nasal mucous membranes were negative for Mycobacterium leprae. Two examinations for Bence-Jones protein and cryoglobulins were negative. Repeat serum protein determination revealed albumin 3.1, globulin 5.2 gm. per cent. Biopsy of the soft palate and skin of the nose revealed hyperkeratosis of the epithelium and moderate pseudoepitheliomatous hyperplasia with a severe chronic inflammatory reaction. (Fig. 2B.) Several leishmania organisms were seen on both sections. Biopsy of the liver revealed focal areas of infiltration by plasma cells, lymphocytes, and a few eosinophils in both the parenchyma and periportal areas. (Fig. 2A.) No leishmania organisms were seen. Sternal marrow aspiration revealed no leishmania organisms and was essentially normal except for a slight increase in eosinophils and a higher proportion of degenerating forms than usually occurs. Attempts to grow the leishmania organisms on NNN media using material from the peripheral blood, bone marrow, liver tissue



2A 2B

FIG. 2. Case I. A, high power photomicrograph of a liver biopsy specimen showing marked cellular infiltration of portal and periportal areas. The cellular infiltrate consists of lymphocytes, plasma cells, eosinophils and macrophages. B, high power photomicrograph of skin of nose showing pseudoepitheliomatous hyperplasia, parakeratosis and marked cellular infiltration by lymphocytes, plasma cells, eosinophils and macrophages. Leishmania organisms were too lightly stained to be seen in the photograph.

and soft palate were unsuccessful. Electrocardiogram revealed sinus tachycardia with occasional extrasystoles and right bundle branch block. Sigmoidoscopy revealed no lesions of the rectosigmoid. Chest x-ray was negative. Fluoroscopy of the chest with a barium swallow revealed slight narrowing at the tracheolaryngeal junction, but the rest of the gastrointestinal series was negative.

Following establishment of the diagnosis the patient was treated with intramuscular injections of fuadin.® The first dose was 1.5 cc., two days later 3.5 cc., and two days later 5 cc., and then 5 cc. every other day for seven doses. This was repeated on two occasions with a week's rest between treatments. At the onset of the first course of treatment the temperature rose to 102°F. and remained elevated for forty-eight hours. There were no further symptoms or reactions. Improvement was noted at the end of the first course of therapy. The hoarseness and dysphagia subsided rather rapidly. The cephalin flocculation test returned to 1+, and the bromsulphalein test showed 12 per cent retention. However, the serum total protein remained essentially unchanged with an albumin of 3.4 and a globulin of 5.4 gm. per cent. The patient had gained 13 pounds. The lesions of the epiglottis regressed completely. The vocal cords were smooth although they remained thickened. A few small nodules still remained on the nose and there was a fine, slightly elevated granulomatous lesion on the soft palate. Because of the persistence of these lesions a second course of fuadin therapy was attempted but following the first injection the patient had severe nausea and vomiting and further treatment was deemed unwise at this time. The patient was examined three months later and, although the marked improvement persisted, the previously mentioned small lesions of the nose and soft palate were still present. The liver and spleen were no longer palpable. Complete blood count and urinalysis were normal. The bromsulphalein test and cephalin flocculation test remained near normal but the hyperglobulinemia persisted (5.6 gm.). Oddly enough, the electrocardiogram, which had previously revealed a right bundle branch block, was now completely within normal limits. Unfortunately, any further follow-up study or therapy of this patient was not possible because he was no longer eligible for care at this hospital.

CASE II. This fifty-seven year old colored man entered Gorgas Hospital on June 3, 1954, for transfer to the Canal Zone leprosarium. The patient resided on a small farm which was in the boundaries of the Canal Zone but some 30 miles from the city of Panama. He was unable to give a good history because of marked hoarseness. His chief complaints were a fungating lesion of the nose, difficulty in speaking and slight dysphagia. Since the patient had been hospitalized at Gorgas Hospital in 1940, the progress of his disease could be followed adequately. A review of his chart revealed that for some ten

FIG. 3. Case II. Frontal view showing the mutilating involvement of the nose and lip. Note the striking similarity in the distribution of the lesions here to Case I.

years prior to 1940 he had noted dysphagia, soreness in the throat and, for a month prior to admission, had difficulty in breathing through his nose. Physical examination at that time revealed the patient to be in apparent good health, weighing 132 pounds, with the only positive physical findings noted limited to the oral cavity. A fungating mass measuring approximately 2 by 2 by 0.5 cm. was found involving the entire soft palate. This was firm but somewhat friable, with secondary ulceration. It was biopsied and microscopically showed only chronic inflammation. The patient's Kahn test was 2+ and the Wassermann test was 3+. He was subsequently discharged from the hospital with a tentative diagnosis of tertiary syphilis with a gumma of the soft palate. He was referred to the Venereal Disease Clinic for therapy but failed to report.

Physical .examination on this admission, fourteen years later, revealed a poorly nourished, chronically ill colored man. He weighed 106 pounds. His temperature, pulse, respiration and blood pressure all were normal. Examination of the head revealed an ulcerative granulomatous lesion involving the upper lip and distal half of the external nose. (Fig. 3.) This involvement extended from the skin on to the mucous membrane of both nostrils to involve the entire mucosa of the turbinates and meati. There was complete absence of the anterior half of the septum. The patient was edentulous. The ulcerative granulomatous lesion involved the entire hard and soft palate. The uvula and central portion of the soft palate were absent. The posterior tonsillar pillars were adherent to the posterior pharyngeal wall. Examination of the nasal pharynx revealed mucopurulent exudate and granulomatous lesions throughout. The hypopharynx and larynx showed thickening and ulcerative granulomatous lesions of the entire epiglottis, arytenoids and ventricles, causing a narrowing of the glottis. The true cords could not be visualized. The tongue and pyriform sinuses appeared normal. There was no lymphadenopathy. The liver was palpable two fingerbreadths below the costal margin and was firm and non-tender. The spleen was enlarged three fingerbreadths below the costal margin and was also firm and non-tender. The remainder of the examination was essentially negative. There was no evidence of other active or healed cutaneous ulcers.

Laboratory findings revealed the following: The white blood count was 7,6000 with a normal differential. There was a normocytic normochromic anemia with a hemoglobin of 9 gm. Urinalysis was normal. Sedimentation rate was 34 mm./hour. Total protein was 7.2 gm. per cent, with albumin 3.45 and globulin 3.75. Liver function studies were normal except for a cephalin flocculation test showing 4 plus in forty-eight hours. Flocculation and complement fixation tests for syphilis were positive. Biopsy of the lesions involving the soft palate revealed chronic inflammation with keratosis and pseudoepitheliomatous hyperplasia. Biopsy of the nasal lesions revealed similar disorders and the presence of leishmania organisms. Liver biopsy revealed periportal and parenchymal infiltration by lymphocytes and plasma cells. Culture of sternal marrow aspirate on NNN media failed to grow any leishmania forms. Chest x-ray and electrocardiogram were normal.

The patient was given a course of fuadin. Following the first two injections his temperature rose to 102°F. which subsequently returned to normal. Because he was not eligible for prolonged treatment at Gorgas Hospital he was discharged without adequate follow-up studies.

COMMENTS

To our knowledge, these are the first cases of American mucocutaneous leishmaniasis reported in the English literature to show unequivocal manifestations of visceral or systemic involve-

ment. Recently Snapper[4] reported a case of mucocutaneous leishmaniasis from South America with visceral manifestations, successfully treated with 2-hydroxystilbamidine. However, the clinical picture was one of severe systemic reaction at the onset, suggesting that the disease may originally have been of the visceral type with secondary involvement of the skin and mucous membranes. In addition, follow-up study of this case revealed a recurrence of severe agranulocytosis and hepatosplenomegaly associated with the ingestion of pyramidon,® barbiturates and optalidon.[17] Since leukopenia had developed in this patient after the administration of chloromycetin® on one occasion in the past, it was Snapper's opinion that the enlargement of the liver and spleen was originally due to drug agranulocytosis which complicated the mucocutaneous leishmaniasis.[17] The systemic involvement in Snapper's case was manifested by fever, hepatosplenomegaly, pancytopenia, hyperglobulinemia and a 4+ cephalin flocculation test. Visceral disease in our patients was evidenced by: (1) moderate hepatosplenomegaly; (2) marked bromsulphalein retention and 4+ cephalin flocculation tests; (3) hyperglobulinemia; (4) cellular infiltration of the liver. It is of special interest to note that while the hepatosplenomegaly subsided and the liver function tests returned to normal following treatment with fuadin in the first case, the serum globulin remained elevated, possibly an indication that parasites were still active in the body. This has been known to occur in kala-azar.[2]

An unusual occurrence was the finding of right bundle branch block by electrocardiogram, which returned to normal following fuadin treatment. The significance of this is not clear and the implication that there was cardiac involvement by leishmania parasites can only be considered speculative, although pathologic changes in the myocardium have been observed in kala-azar.

Although the clinical picture of cutaneous and visceral leishmaniasis is fairly well established and agreed upon, there is a great deal of confusion in the literature regarding the mucocutaneous variety. Kirk,[5] in reporting from the Sudan, compared the oro-nasal leishmaniasis of that area with the American variety (espundia) and described the following differences: (1) There is a history of primary lesions in the American disease, similar to oriental sore, which heal leaving characteristic scars, and are followed months or years later by the mucous membrane lesions. In the Sudan condition primary cutaneous lesions are rare and inconspicuous, if they occur at all. (2) There is no involvement of the abdominal viscera in espundia, while in the Sudan variety disease of the liver and spleen is common. In a later paper[6] Kirk speculated that even though no cases had yet been reported it was probable that systemic infestation with the parasite occurred in American mucocutaneous leishmaniasis; he reasoned that the parasites could not invade the nasal pharynx many years after the primary lesion had healed without systemic migration. Our patients confirm his suspicion. In this same communication[6] Kirk also hypothesized that leishmania infestations went through a specific course of evolution in humans, characterized by three stages: (1) Primary, in which there appeared cutaneous sores at the site of inoculation which tended to heal spontaneously. (2) Secondary, in which a generalized infestation occurred, kala-azar. (3) Tertiary, in which there were cutaneous and mucocutaneous lesions. Fox[7] has written an excellent review article on American leishmaniasis, with personal observations on cases he had seen in Brazil. He believed that the cutaneous and mucous membrane involvement were simply variants in the manifestations of the same disease, the mucosal lesions being caused by metastasis or autoinoculation. He reviewed some of the statistics on the relative frequency of the mucosal type, estimating it to be from 10 to 20 per cent. In none of the cases that Fox reviewed was there any evidence of visceral disease, and this fact made him believe that the metastatic theory of the origin of the mucous membrane lesion was improbable.

In comparing the observations made by Kirk and Fox, it is difficult to decide which form of mucocutaneous leishmaniasis our cases most resemble. Certainly the visceral involvement is typical of the Sudan variety but the history in the first patient of primary lesions which healed would classify this as a typical case of espundia.

It is of interest to review the history of leishmaniasis in Panama. Pure visceral leishmaniasis has never been reported, and the occurrence of mucocutaneous leishmaniasis has been reported only once previously.[8] The description of the lesions in that case were typical and the organisms were found by biopsy. Unfortunately,

further investigation and follow-up were inadequate because the patient was lost from observation. Certainly, the report of only three cases of mucocutaneous leishmaniasis in the history of the Canal Zone emphasizes the rarity of this condition in Panama. Of special interest is the fact that our first patient had spent the past eleven years of his life in the larger cities of Panama, during which time he traveled into the interior of the Republic only briefly while working for the U. S. Government in World War II, but never actually lived in the "bush." Classically, this disease occurs in men working in the forest.[2]

In contrast, cutaneous leishmaniasis is not uncommon in Panama and several papers describing the clinical and pathologic characteristics have been published from the Canal Zone.[9–15] All of these cases responded well to therapy so that the natural course of the disease in this area is not known. However, on trips into the interior ("bush") typical scars of the disease have been seen, indicating that the lesions do heal without therapy. This suggests, too, that the incidence of cutaneous leishmaniasis in Panama is greater than the forty-seven cases that have been reported thus far.[14] Calero and Johnson,[14] in reporting twenty-five cases from Panama, suggested that the fact that their patients came from various localities within the country rather than one area alone verified the impression that the disease has a generalized distribution in the country.

Just as there has been much confusion in the literature concerning the clinical descriptions of mucocutaneous leishmaniasis, so has there been controversy over the causative agent. Fox[7] believed that both the cutaneous and the mucosal types were caused by L. braziliensis and cited the immunologic investigations of Noguchi, in which he found three distinct serologic types—L. donovani, L. tropica and L. braziliensis. Ash and Spitz,[16] on the other hand, believed that the organism which caused skin and mucous membrane ulcers in the New World was identical with or a variant of the same species of leishmania responsible for oriental sore and should be called Leishmania tropica var. Leishmania americana. In the original cases of dermal leishmaniasis reported by Darling from Panama, the causative agent was considered to be L. tropica. In a recent paper on the histopathology of the disease reported from Panama by Thornburgh et al.[15] L. braziliensis was considered the offending parasite. In our first case the pathologists could identify the parasite only as either L. tropica or braziliensis Unfortunately, we were unsuccessful in culturing any of the organisms on NNN media even though we inoculated material obtained from the palate, liver, bone marrow and blood, so that further immunologic studies could not be carried out.

The treatment of leishmaniasis has been accomplished for the most part by the use of antimony and diamidine compounds. Although the antimony derivatives have been successful in the large majority of cases, the mucous membrane lesions are frequently resistant to these drugs.[7] This appeared to be the case in one of our patients; although he was markedly improved by the fuadin injections, the lesions of the mucous membranes did not completely disappear. It could be argued that our patient did not get a sufficient amount of fuadin to cure him completely but we believed that the vomiting was sufficiently severe to portend serious antimony toxicity. Unfortunately, the patient was no longer eligible for treatment in the Canal Zone under government ruling, and we were unable to try a course of stilbamidine therapy.

CONCLUSIONS

The demonstration of hepatosplenomegaly accompanied by abnormal serum proteins, altered liver function tests and cellular infiltration of the liver in two cases of "espundia" clearly establishes the fact that visceral involvement may be an integral part of American mucocutaneous leishmaniasis. We feel justified in considering our cases the "missing link" between the Old World variety and the American counterpart of the disease, since systemic manifestations were never before demonstrated in the latter. These findings have probably not been searched for adequately in the past. We believe, then, that the separation of two distinct forms of mucocutaneous leishmaniasis, based on clinical features alone, is invalid.

In view of the seriousness of this disease and the failure of some cases to respond to antimony compounds, it is recommended that diamidine therapy be considered for use in this disease. The successful treatment of a case of mucocutaneous leishmaniasis by 2-hydroxystilbamidine reported by Snapper[4] suggests that this drug merits further trial in this disease.

SUMMARY

1. Two cases of American mucocutaneous leishmaniasis with visceral manifestations are presented. These are the second and third cases of the mucosal variety of the disease to have been reported from Panama, emphasizing the rare occurrence of this disease in this area.

2. Systemic involvement was manifested by hepatosplenomegaly, hyperglobulinemia, abnormal liver function tests and cellular infiltration of the liver.

3. It is believed that the separation into two distinct forms of mucocutaneous leishmaniasis (Sudan and American) based on clinical features is invalid.

4. Antimony treatment (fuadin) succeeded in ameliorating the disease without producing a complete cure in one case.

5. It is recommended that 2-hydroxystilbamidine be given further trial in the treatment of mucocutaneous leishmaniasis.

ADDENDUM

Since the preparation of this manuscript we have seen a third case of mucocutaneous leishmaniasis with certain systemic manifestations. Thus to date, a total of four cases of mucocutaneous leishmaniasis have been reported from Panama.

Case Report. A seventy year old Panamanian was admitted to Gorgas Hospital in May, 1954, having been transferred from the Canal Zone leprosarium, Palo Seco. He had been a patient at the leprosarium for four years because biopsies from his nose had been reported as showing M. leprae. However he failed to show any response to the usual therapeutic regimen and it was the opinion of the chief of service at the leper colony that the patient should have another biopsy and be re-evaluated. There was no family history of leprosy nor was there any known contact with that disease. The patient's only complaint was of severely painful sores in his nose. On admission, physical examination revealed a small cachectic man with a wide, flat nose and an area of erythema over the upper lip. The entire nasal septum was absent. The soft palate was covered with red granular tissue that extended back into the pharynx. There were several small lymph nodes in the left submandibular region. The chest was markedly emphysematous. Examination of the abdomen at the time of admission revealed no abnormalities, but in subsequent examinations the liver was felt two fingerbreadths below the costal margin. There was no splenomegaly. Laboratory findings were as follows: white blood count 3,300 with 32 polymorphonuclears, 56 lymphocytes, 12 eosinophils. Red blood count was 3,960,000, hemoglobin 11.5 gm., hematocrit 34 per cent. Sedimentation rate was 32 mm./hour. Stools were positive for occult blood and the ova of Uncinaria and Trichocephalus. Bromsulphalein test revealed 14 per cent retention at the end of forty-five minutes. The prothrombin time was 64 per cent, cephalin flocculation test was 2+, thymol turbidity 4.6 units. Total proteins were 6.9 gm. per cent with an albumin of 2.8 gm. per cent and globulin of 4.1 gm. per cent. Liver biopsy was normal. Biopsy of nasal mucosa revealed leishmania organisms. An electrocardiogram was within normal limits. A gastrointestinal series showed no abnormalities. The patient was given three courses of fuadin one month apart. His nasal lesions showed considerable clearing and he was free of pain. He gained 14 pounds. At the time of discharge the liver function studies and the albumin-globulin ratio returned to normal.

Comment: This case presented a picture almost identical to the two other cases with the exception of the absence of splenomegaly. Interestingly enough all three patients were originally thought to have leprosy, illustrating the ease with which mucocutaneous leishmaniasis can be confused with Hansen's disease; leprosy is not uncommon in Panama and most physicians in this area are familiar with the clinical picture. In addition to the hepatomegaly and the altered liver functions, this third patient also had leukopenia, a common finding in classic visceral leishmaniasis (kala-azar). The anemia was probably a result of infestation with hookworm.

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Psittacosis in Northern New Jersey

Human and Bird Transmitted

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PSITTACOSIS is rarely encountered by the practicing physician. Its symptoms are similar to those of influenza and unless the patient gives a history of contact with psittacine birds nothing would be present to alert the practitioner to the presence of an infection other than influenza.

The increasingly widespread popularity of parakeets and other psittacine birds may change this. Wholesale shipments of psittacine birds have included birds from areas of infection, with consequent introduction of the disease to many flocks. Reports on this disease are increasing in the literature. In some areas of this country psittacosis is recognized as an occupational disease.[1]

The Surgeon General[2] recently made an appeal for prompt reporting of all human cases of such illness and of illness among birds to public health authorities so that the origin of the infection can be investigated and appropriate control measures instituted to prevent further spread of the disease. In the cases presented herein, prompt reporting of a recognized case led to establishment of an already suspected source of infection and to the discovery of an epidemic.

CASE REPORTS

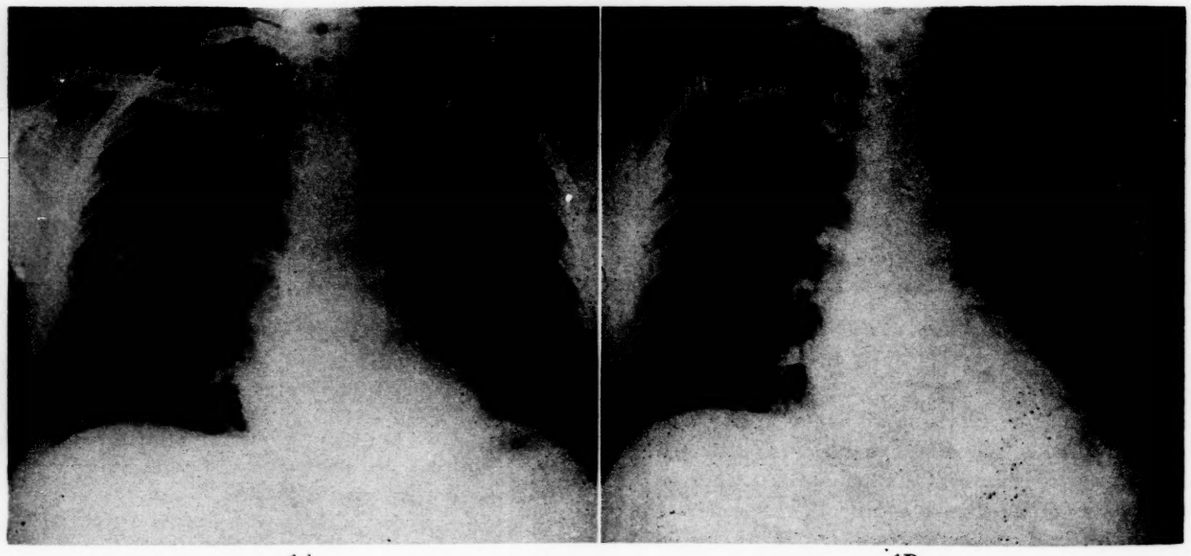
CASE I. On April 21, 1954, L. L., a fifty-one year old saleswoman, presented herself in an extremely weakened condition with a temperature of 103.2°F. and a chief complaint of constant, intolerable, stabbing frontal and occipital headache of several days' duration. The headache, according to her statement, was, in intensity and character, unlike any headache she had ever had except for an episode a few weeks previously when she had had a similar attack with temperatures ranging up to 103.2°F. and had been confined to bed. At that time she had also suffered chest pains and felt extremely weak. The physician whom she consulted told her she had a very severe attack of grippe and administered one and a half million units of penicillin daily for several days. After a week's confinement in bed, she attempted to return to work but was unable to perform her duties. Her condition became worse, culminating in the attack presently being discussed.

The background history was non-contributory and not suggestive of any connection with her present illness. The patient has been in the menopausal state for two years. She had had a left ovariectomy in 1932, a mass removed from her intestine in 1948, and in 1951 a fibroma had been removed from the ulnar nerve.

The family history was also non-contributory. At present she and her husband, a retired white collar worker, live in the country rather isolated from other dwellings. They have no pets and have no contacts with barnyard animals.

Physical examination revealed a feverish-looking woman in apparent pain with a temperature of 103.2 °F. The blood vessels of the pharynx were injected. On percussion of the chest barely perceptible impairment of resonance was noted between the fourth and sixth ribs on the left side with slightly sharper expiratory sounds over the same area. The spleen was soft, palpable and enlarged. Blood pressure was 145/102. The eye grounds, ears and extremities disclosed no deviation from normal. The skin was clear. There was a slightly enlarged thyroid. No lymph glands were palpable. Reflexes were physiologic.

The patient was questioned intensively as to her contacts and activities. She revealed that the duties at her place of employment included daily cleaning of birdcages in which parakeets were kept and that a month prior to her illness a parakeet had died. With this further information, psittacosis was considered and a chest x-ray was obtained. The positive findings were confined to the left upper lung field. This consisted of fan-like distribution of an infiltrate which resembled interstitial pneumonia. (Fig. 1A.) It was not associated with left hilar enlargement. The lateral film (which is not reproduced here) did not demonstrate the exact location of the interstitial infiltrate, but there was suspicion that it was located posteriorly, most likely in a posterior lobular division of the left upper lobe. A slight increase in the peribronchial markings at the right base was noted but it was



1A 1B

FIG. 1. A, interstitial pneumonitis in left upper lung field at crossing of second and fifth ribs. B, complete resolution of process shown in A.

thought that this was not related to the original process. The remaining lung fields were completely clear. The infiltrate was not associated with any pleural reaction that could be demonstrated on the x-ray. The findings were typical of psittacosis.[3]

It was decided to start immediate intensive antibiotic therapy and not to wait for the report of the serum agglutination test, the result of which might be questionable in view of the fact that the patient had previously received large doses of penicillin. The patient was given 250 mg. of terramycin® four times a day for one day, then aureomycin, 250 mg. four times a day, and complete bed rest was ordered. She was kept on this regimen for ten days. The prolonged application of aureomycin was to prevent any relapses because it was assumed that when the patient was first seen by one of the authors (E. S.), relapse was present, the original illness having started several weeks earlier.

Her headache receded the following day and she became afebrile and free of headache on the third day. It is significant that the serum agglutination titer was very low in the beginning, showed an increase only after many weeks, and then gave entirely negative results. (Fig. 2.)

A repeat x-ray was made on May 13, 1954, or approximately three weeks later. At this time, complete resolution of the interstitial type of pneumonitis was demonstrated (Fig. 1B) and the chest was considered normal except for increased markings at the right base which, in the opinion of the examiner, had not significantly altered. At the time of this second examination, the patient had improved considerably.

Boyd[4] has described this interstitial type of pneumonitis as involving the interalveolar tissues as well as the alveolar walls, and as being located predominantly in the lobular segments of the lung. It was felt that this was a classical type of psittacosis infiltrate. This disease, of course, manifests itself in many varied pulmonary forms.

CASE II. R. L., a fifty-three year old retired civil service employee, the husband of the patient just described, began to complain on April 29 of slight stabbing chest pains, exhaustion with excessive perspiration and slight headache after light work. He had no fever, cough or any other symptoms. His past history included a brain concussion suffered in 1951, which left him with frequent headaches. Otherwise, the history was non-contributory. He transported his wife to and from her place of employment daily but, according to his statement, never entered the premises. He had been previously instructed to keep an accurate record of his temperature, and blood was taken from him for serum agglutination test at the same time it was taken from his wife.

Physical examination did not reveal any deviation from the normal. X-rays taken the first day of his complaints did not show any significant heart or lung change. Since the x-rays seemed to be completely negative for pulmonary involvement, the patient's complaints did not appear to have any connection with his wife's illness. On May 10, the results of the first serum agglutination test were available and the titer was significantly higher than that of his wife. In view of the scanty findings, it was thought that an error had been made in labelling the specimens by transposing the labels from husband to wife and vice versa, thereby giving the apparently contradictory results. A new blood test was made immediately, the results of which (available on May 18) gave essen-

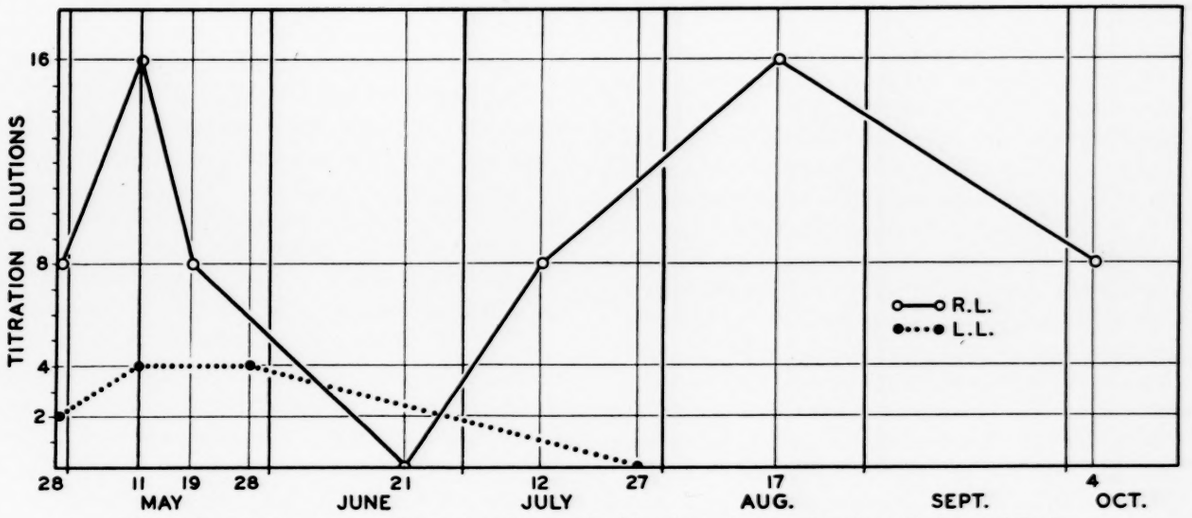


FIG. 2. Graph showing results of titration on the blood specimens of patients L. L. and R. L.

tially the same result as the first This finding was interpreted as signifying that the husband was also infected and the low titer of the wife was due to scanty antibody formation caused by the prior administration of penicillin.

Antibiotic therapy was instituted and rest at home without strict bed confinement was ordered. The patient remained on this regimen from May 19 to June 4. In spite of these therapeutic measures the patient continued to complain of chest pains and extreme weakness for a week, after which he gradually improved. The serum agglutination test gave negative results two weeks after cessation of treatment. At the beginning of July the patient moved to new living quarters and the moving was connected with some unusual physical labor. His complaints increased, the liver became palpable under the costal margin and the titer showed a significant increase. (Fig. 2.)

COMMENTS

The wife showed a much more satisfactory response to treatment than her husband who still complains of occasional stabbing chest pains and easy fatigability. Neither patient had any expectoration. Mucus taken from the husband's mouth in an attempt to isolate the virus proved negative.

An epidemiologic investigation made by the State Department of Health confirmed that the initial patient, L. L., had been in rather intimate contact with birds (parakeets) which were infected with the psittacosis virus. This investigation showed that birds suspected of harboring the virus had been sent to the variety chain store where the patient had been employed to take care of parakeets, to sell them and, most important, to clean the cages.

Investigation further revealed that this store had received shipments of birds from a bird seed and bird supply company located in a large metropolitan area nearby (New York City). This company had been implicated in connection with cases of psittacosis in nearby states (Pennsylvania and New York). The company obtained birds from Florida, Texas and California. Infected birds had been traced to this company by other investigators in other reports.[5] Tests subsequent to the findings in patient L. L. did in fact reveal that birds harboring the virus of psittacosis were on sale. An embargo was imposed, the public notified and suspect and contact birds destroyed.

It is interesting to note that, entirely independent of the findings in this case, an epidemiologic investigation had been undertaken by the New Jersey State Department of Health. The investigation revealed that on April 9 a shipment of infected birds from Texas had been received at the New Jersey depot of the aforementioned bird seed and bird supply company. This shipment, involving approximately 28,000 birds, was embargoed by one of us (O. S.) on the basis that the birds had entered New Jersey illegally, that dead birds requested for examination upon the original investigation were destroyed by the management before examination could be made, and that some of the birds appeared to be ill. Some birds were subsequently obtained for examination and these were submitted to the State Poultry Pathology

Laboratory at Rutgers University where the diagnosis of psittacosis was made on April 28. Confirmation of the presence of psittacosis virus was made by the company's New Jersey depot from a report by the Communicable Disease Center, Montgomery, Alabama, where several lots of birds had been sent for virus isolation.

It is evident from the epidemiologic investigation that the original case of psittacosis in the woman patient was the result of intimate contact with infected birds. It is also quite probable that the husband of this woman, through intimate contact with his wife, was exposed and did contract the virus during the acute phase of the wife's illness. Thus there is a strong indication that the original contact was due to birds and the subsequent case to human contact.

Other outbreaks of psittacosis occurred in scattered parts of Northern New Jersey, the cause of which could be traced to one central source of infected birds. The disease was also disseminated by human transmission.

Intense headache and great weakness in the presence of only minimal pulmonary involvement demonstrable by roentgenogram is characteristic of psittacosis.[6] According to Green,[7] known exposure to infected birds before onset of the illness, the clinical course and the serologic changes establish the diagnosis of psittacosis. Antibiotic therapy given in the early stages of the disease may suppress antibody formation completely or in part, and isolation of the agent from the sputum and blood may become impossible.[8–10] In our patients, the penicillin effect upon the wife gave rise to confusion in the beginning of the investigation. The wife gave evidence of a low titer with distinct clinical manifestations of psittacosis; the husband gave evidence of a high titer with only subjective symptoms.

ADDENDUM

Since this article was submitted for publication, the electrocardiogram of one of the patients (R. L.) revealed myocarditis. One of the authors (E. S.) recently observed another case of psittacosis in a woman, K. W., who contracted the disease from the same source. The electrocardiogram of K. W. also revealed myocarditis. This is noteworthy, as myocardial involvement is rare in psittacosis.

Acknowledgment: The authors wish to express their sincerest thanks for the efforts of Mr. Clarence Bunting and Mrs. Eleanor E. Thomas, New Jersey State Department of Health, Division of Laboratories, and Dr. Clarence Manziano, Practicing Veterinarian, Jersey City, New Jersey, formerly Veterinary Epidemiologist, New Jersey State Department of Health.

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Postnephrectomy Renal Failure in a Patient with a Normal Preoperative Blood Non-Protein Nitrogen*

T. English McGeachy, M.D., William Bloomer, M.D. *and* Arthur J. Merrill, M.D.
Atlanta, Georgia

Often the blood NPN or urea is the only study made of renal function before a damaged kidney is removed. In most instances the patient with a normal blood urea seems to do well. The blood NPN of the patient to be described was normal, yet it appeared that she might die from renal tubular insufficiency following operation.

CASE REPORT

The patient was a seventy-seven year old white female who in 1941 was found to have pyuria. Aside from non-union of a femoral fracture, she was in fair health. Despite treatment with citrasulfa® and gantrisin® infection persisted, with occasional febrile and symptomatic exacerbations.

She was admitted to the hospital July 29, 1952, with no complaints. Physical examination was essentially normal. The skin had a good turgor. The arterial pressure was 170 systolic, 90 diastolic. The blood count was normal and blood NPN on July 30th was 28 mg. per 100 cc. and the creatinine 1.2 mg. per cent.

Cystoscopic examination was performed. Turbid urine was obtained from the right kidney and clear urine from the left. The right kidney was moderately ptotic and its pelvis was dilated. Nephrectomy was decided upon.

During the operation, August 1st, the patient received 1,500 cc. of 5 per cent dextrose in distilled water and 500 cc. of whole blood. Blood pressure was normal throughout the operation. In the afternoon after the operation she received some sedative and 1,000 cc. 5 per cent dextrose in distilled water. During the night she was restless but rational. On August 2nd 2,000 cc. of 5 per cent glucose in distilled water were given and the patient's urinary output was 965 cc. That night she was very restless and at 7:00 A.M. the following day she voided involuntarily, was drowsy and would not eat. Only after repeated questioning would she respond. The blood NPN was 25, creatinine 1.6 mg. per 100 cc.; fluid intake, 2,170 cc., mostly 5 per cent glucose in distilled water; output uncertain but more than 380 cc.

On August 4th she was more drowsy and had Cheyne-Stokes respirations. Blood NPN was 33 mg. per 100 cc., CO_2 combining power (bicarbonate) 22.7, chloride 74, sodium 103, potassium 3, mEq./L. An infusion of 1,300 cc. 5 per cent glucose in distilled water had been started, but after her blood reports became known this was stopped and she was given 400 cc. 5 per cent NaCl with KCl, 1 gm., intravenously. Within four hours she was distinctly more responsive.

On the following morning her blood bicarbonate was 21.8, chloride 87, sodium 123, K 3.3 mEq./L., sugar 132 mg. and NPN 36 mg./100 cc. Respirations were deep and labored, tongue wrinkled, skin inelastic and putty-like. "Fingerprint" edema was present and the muscles were flabby and felt like bags of water. At 1:00 P.M. 300 cc. of 5 per cent NaCl were given and at 7:00 P.M. were repeated. Despite low blood sodium and chloride, she excreted 1,720 cc. of urine containing 54 mEq. of Na and 50 mEq. of chloride. She was given only half-normal saline to drink.

That night she ate well, slept well and was stronger and more alert. Blood analysis revealed NPN 33 mg. per cent, bicarbonate 23.6 mEq., chloride 104, Na 142 and K 2.6 mEq./L. Fifteen hundred cc. of normal saline were

* From The Department of Medicine, Emory University Medical School, Atlanta, Ga.

TABLE 1

Date	Intake				Output (urine)				Blood				
	Water (ml.)	NaCl (gm.)	$NaHCO_3$ (gm.)	KCl (gm.)	Vol. (ml.)	Na (mEq.)	K (mEq.)	Cl (mEq.)	NPN (mg. %)	HCO_2 (mEq.)	Cl (mEq.)	Na (mEq.)	K (mEq.)
7/31/52	....	....	..	...	....	...	..	...	28	....	...	...	..
8/1/52	1000	....	..	...	420	...	..	...	..	....	...	...	.
8/2/52	2185	....	..	...	965	...	..	...	..	....	...	...	..
8/3/52	2230	....	..	...	380+	...	..	...	25	....	...	...	...
8/4/52	700	20	..	1	313	...	..	...	33	22.7	74	103	3
8/5/52	1045	30	..	1	495+	34	..	50	36	21.8	87	123	3.3
8/6/52	2175	17.5	..	3	250+	...	..	...	33	23.6	104	142	2.6
8/7/52	2570	15.2	..	2.4	1785	140	25	185	33	23.2	99	129	3.6
8/8/52	2505	17.8	..	4	470+	...	..	...	..	....	...	...	...
8/9/52	1770	10.5	2	4	2675	303	57	326	24	30.3	94	132	4.6
8/10/52	2010	14.0	..	4	1726	...	..	...	..	....	...	...	...
8/11/52	1650	12.5	..	4	2655	...	..	...	..	....	...	...	...
8/12/52	2275	15.2	..	4	2398	...	..	...	38	23	96	137	4.6
8/13/52	1800	11.3	2	2	1445	...	..	...	..	....	...	...	...
8/14/52	1825	11.5	2	2	1635	88	35	90	36	29	98	137	4.2
8/15/52	1900	?	..	...	1040	...	..	...	..	....	...	...	..
8/16/52	1575	?	..	...	1040	...	..	...	39	32	98	130	4.1
8/17/52	1775	?	..	...	1875	...	..	...	..	....	...	...	...
8/18/52	....	....	..	...	.....	...	..	...	32	29	93	133	4.0

administered with 2.6 gm. of KCl intravenously. KCl was given by mouth, 0.3 gm. every 30 minutes, for nine doses.

The patient's condition improved steadily until August 10th when her sensorium was essentially normal except for occasional involuntary urination. On August 15th her condition was excellent and she was discharged four days later. Pus was present in her urine but this finally cleared with chemotherapy. Treatment during the latter part of the hospital stay and blood chemical values are summarized in Table 1.

At present the patient seems well but still has pyuria occasionally.

COMMENT

This patient evidently had renal tubular insufficiency without appreciable glomerular insufficiency. This condition may not be accompanied by nitrogen retention. The inability of the distal convoluted tubules to form ammonia and exchange H^+ ions for sodium resulted in a chronic acidosis which in turn produced a loss of cations (Na^+ and K^+). It is unlikely that such striking cation depletion could have occurred within forty-eight hours; it is probable that her blood sodium and possibly potassium levels were low before operation. Her response to electrolyte administration (sodium, potassium, chloride and bicarbonate) indicates that cation depletion was her chief problem. Pyelonephritis, which damages the tubules initially and involves the glomeruli late, could account for her abnormal tubular function with essentially normal blood NPN.

A crude estimation of glomerular function is usually represented by the blood NPN. An impaired phenolsulfonphthalein test indicates diminished function of the proximal convoluted tubules, while the concentration test measures distal tubular activity. In some instances any one of these functions may be diminished in the presence of normal activity of either or both of the other two. Proper evaluation of the patient's renal physiology may necessitate testing each of these three segments of the nephron. Failure to do so may result in inadequate preparation of the physician for serious postoperative complications, as in the case of this patient. If the kidneys are unable to concentrate, serum sodium, chloride and bicarbonate should be measured even when the blood NPN is normal. In the presence of adequate glomerular filtration, as indicated by a normal blood NPN, adjustment of electrolytes is not difficult especially if the physician is forewarned.

SUMMARY AND CONCLUSIONS

A case report is presented in which the blood NPN was normal both before and after unilateral nephrectomy. However, tubular insufficiency with electrolyte depletion which could have resulted fatally became evident during the postoperative period.

Blood NPN determination alone may not be adequate to estimate renal function prior to nephrectomy.

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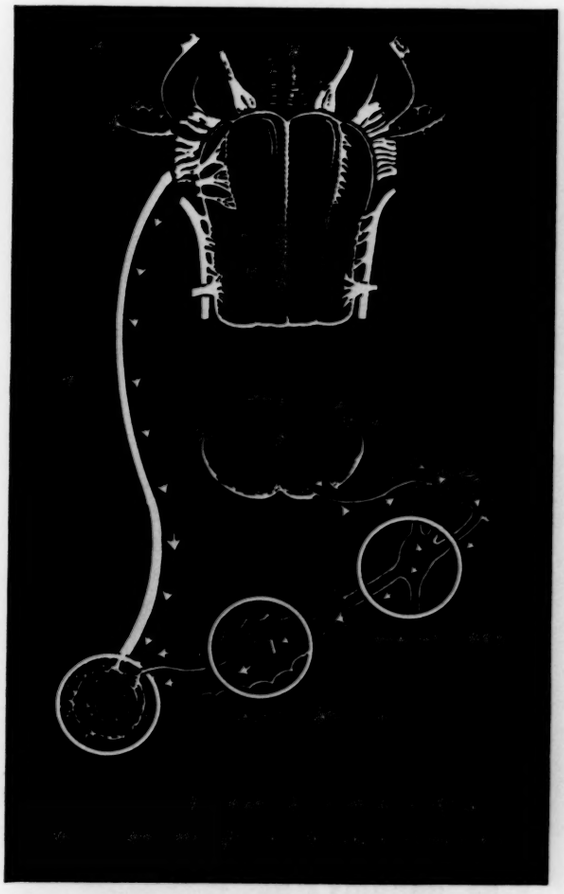

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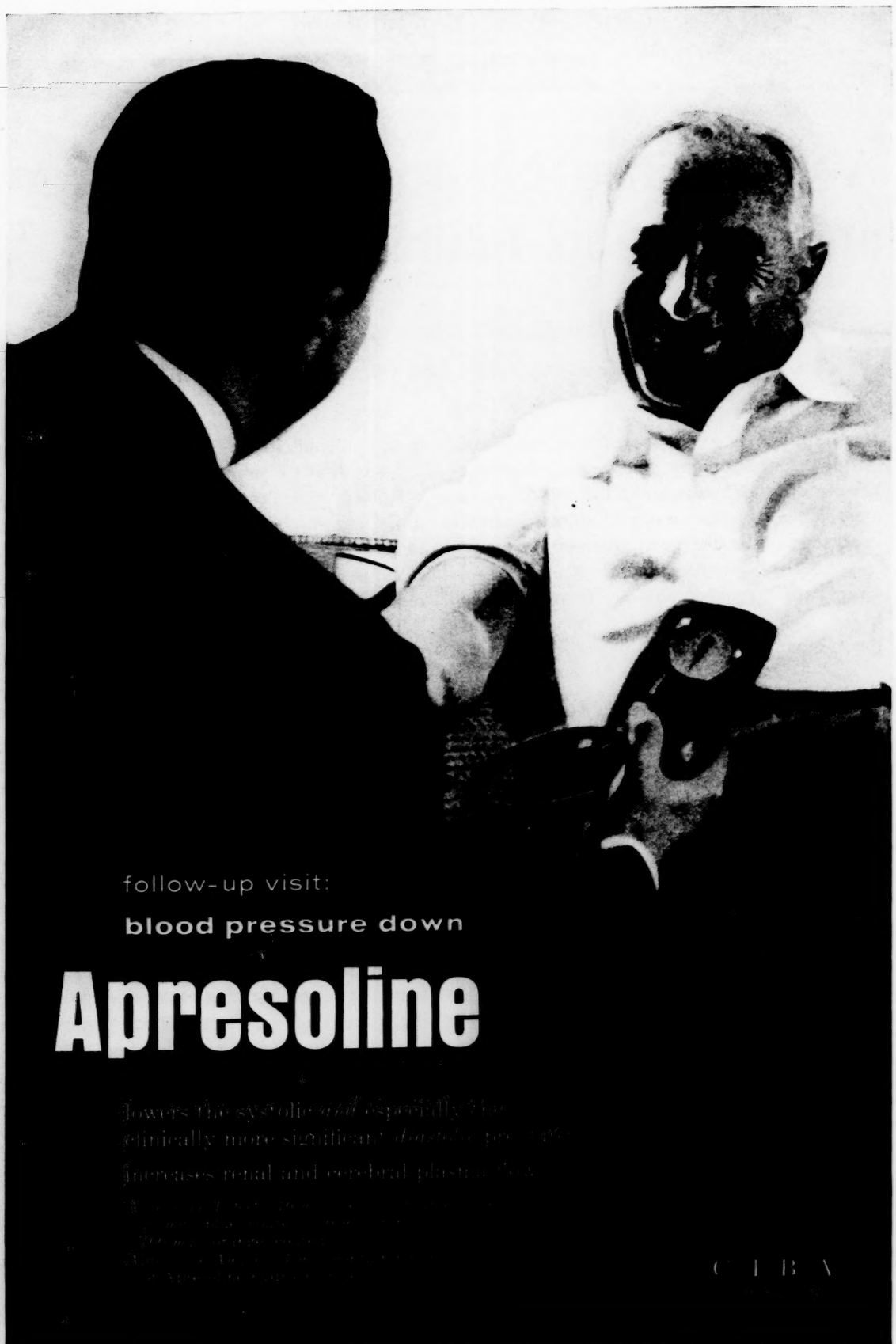

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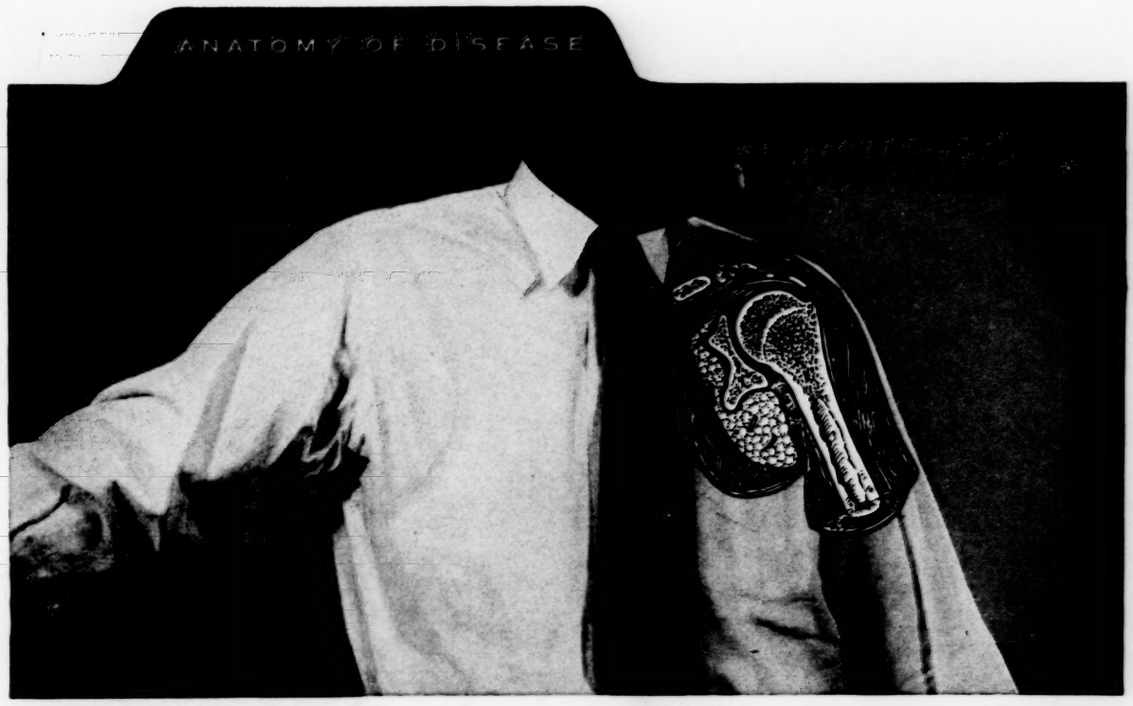

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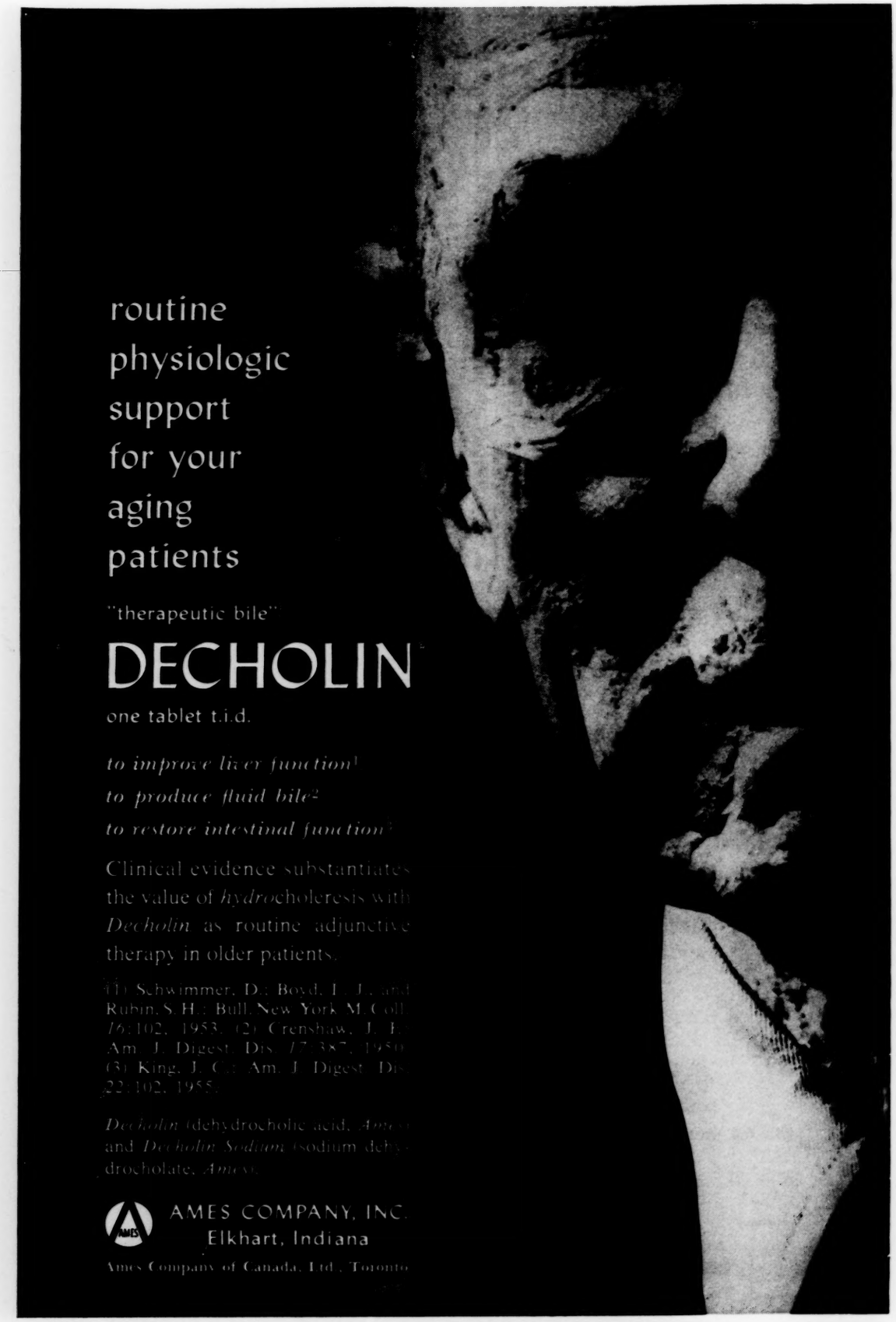
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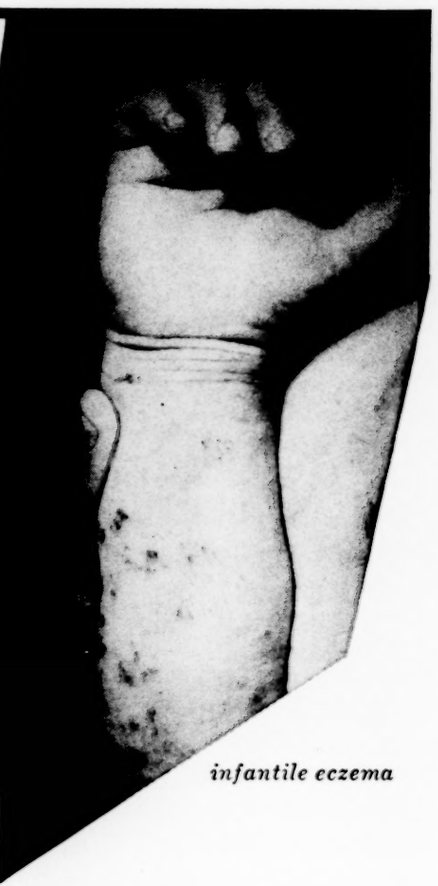

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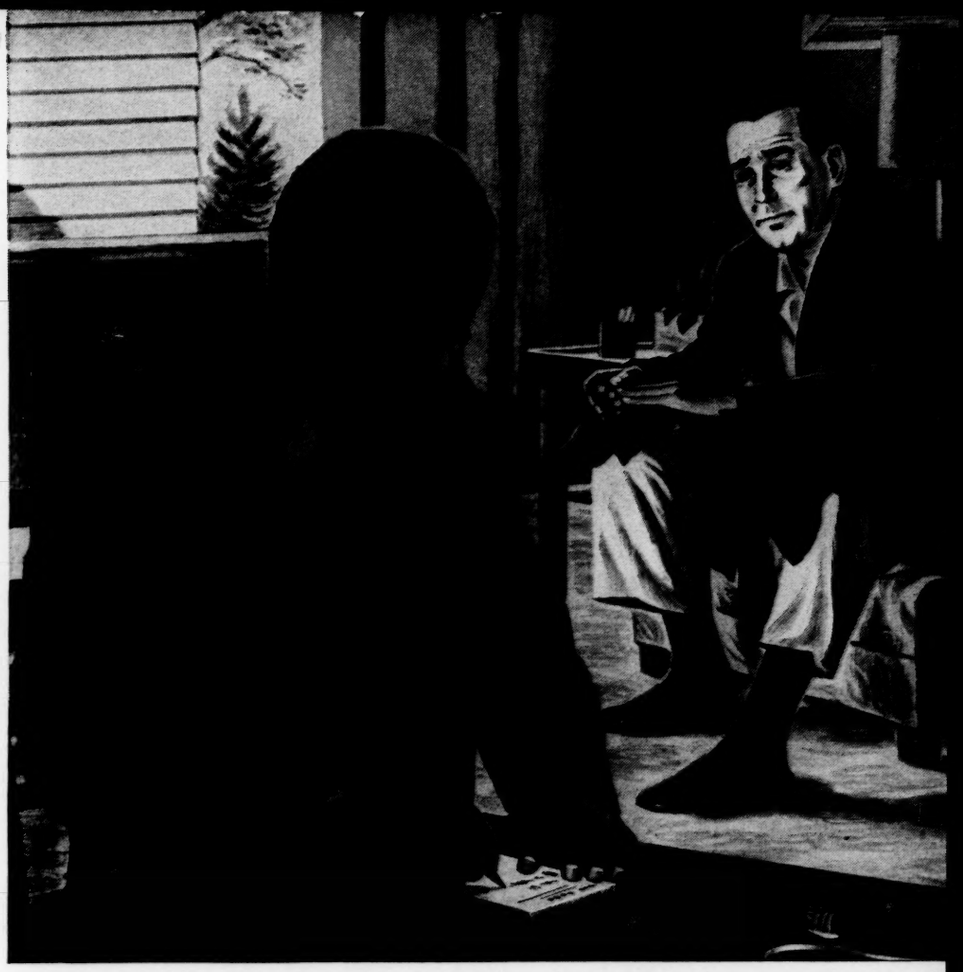

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REFERENCES:

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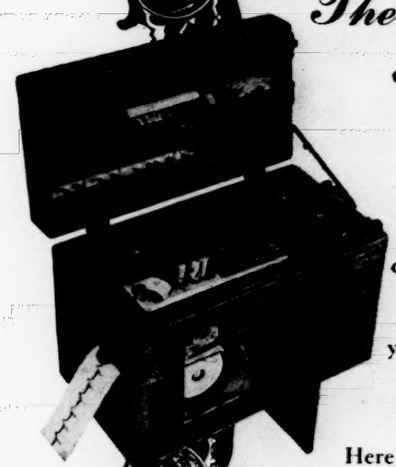

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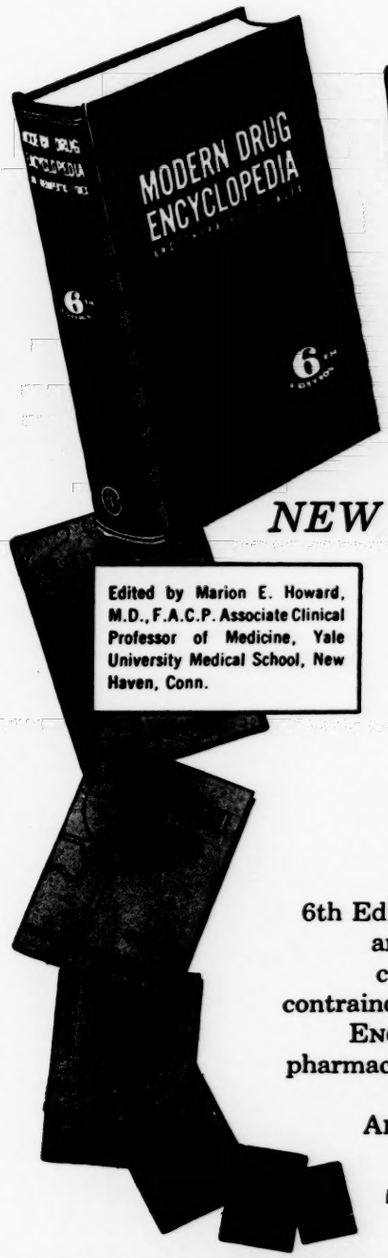

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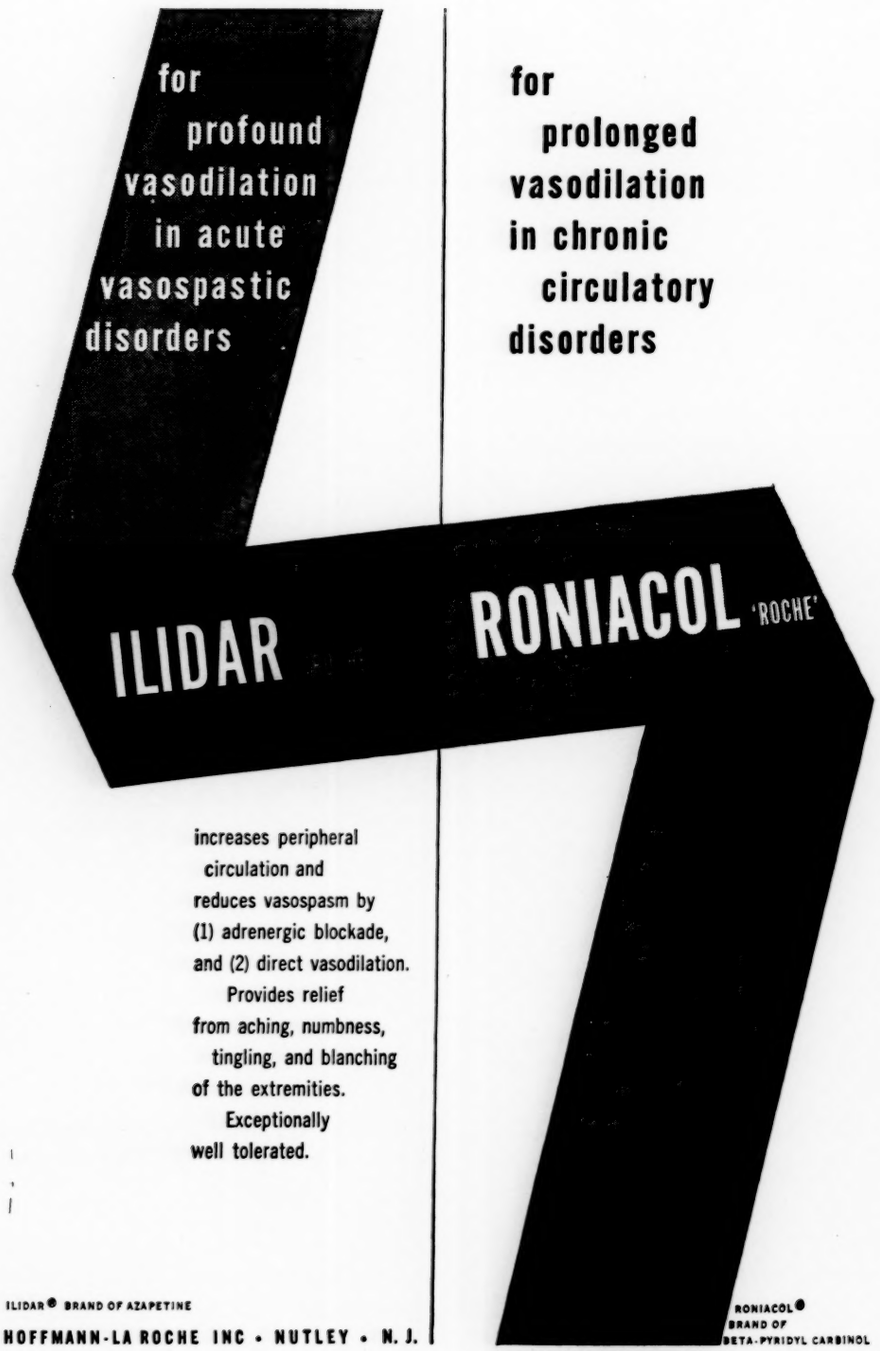
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